Activation of Protein Kinase Cε in Merkel Cell Polyomavirus-Induced Merkel Cell Carcinoma

Merkel cell carcinoma (MCC) is a rare neuroendocrine tumor of the skin\(^1\) that has a maximal incidence in elderly and immunocompromised patients. As the number of elderly and immunocompromised patients continues to rise, the incidence of MCC is rising as well. Radiation, chemotherapy, surgery, and now immunotherapy have been used against MCC.\(^2\) While advances have been made, a better understanding of its signaling is required for cure of MCC.\(^3\) We found that activated protein kinase Cε (phospho-PKCε) is highly associated with Merkel cell carcinoma-associated polyoma virus (MCPyV), and that this pathway might be a druggable target for polyoma-induced malignancy.

Methods | In a retrospective cohort-based study, 11 MCC specimens obtained from different Emory University School of Medicine patients were evaluated. Specimens were previously histologically diagnosed as MCC by dermatopathologists from the Departments of Dermatology and Pathology of Emory University School of Medicine; institutional review board approval was waived.

Immunohistochemical analysis with antigen retrieval was performed. Slides were stained with primary antibodies against PKCe (PKCe) (EPR14822; ab124806; Abcam Inc), phospho-PKCe (phosphorylated S729, ab163387; Abcam Inc), or MCPyV large T-antigen (CM2B4, sc-136172; Santa Cruz Biotechnology Inc) diluted 1:100 with Dako EnVision and DualLink System-HP (Dako North America Inc) for 40 minutes at room temperature. Slides were washed 3 times with wash buffer. Rabbit or mouse polyclonal or monoclonal antibodies were used, and slides were applied with Rabbit-on-Rodent or Mouse-on-Mouse HRP-Polymer (Biocare Medical), respectively, for 30 minutes. Slides were washed 3 times with wash buffer. Antibody binding was detected using the Liquid DAB+ Substrate Chromogen system (Dako North America Inc) applied to slides for 2 minutes. All slides were signed out by 2 evaluators; immunohistochemical staining was graded in accordance to a subjective, consensual scale from 0 to 4 (0, negative; 1, positivity varying from 1%-25%; 2, positivity varying from 26%-50%; 3, positivity varying from 51%-75%; 4, positivity varying from 76%-100%).

For statistical evaluation, a 5% significance level was used. ANOVA (analysis of variance) testing was used to measure variation within the results and compare data between groups. We applied the Bonferroni posttest to increase the precision of the ANOVA (GraphPad Prism v6).

Results | Our samples were from 5 women (45.45%) and 6 men (54.55%). Patient mean (SD) age was 73 (3) years (median, 71 years; range, 50-90 years).

Table. Demographic Information and Immunohistochemistry Results for 11 Merkel Cell Carcinoma Cases

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Grading From 0 to 4</th>
<th>Phospho-PKCe</th>
<th>PKCe</th>
<th>MCPyV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/60s</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2/F/80s</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3/M/70s</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4/M/60s</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>5/M/70s</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6/M/70s</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>7/M/90s</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8/F/50s</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>9/F/70s</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>10/F/80s</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>11/M/80s</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male; MCPyV, Merkel cell carcinoma associated polyoma virus; PKCe, protein kinase Cε.

Figure. Merkel Cell Carcinoma Specimen, Case 4

A Original magnification ×40  
B Original magnification ×40

A, Hematoxylin-eosin, and (B) positive staining for activated protein kinase Cε.
Phospho-PKCε seems to be positive in 8 of 11 MCC specimens (73%) (grafting mean [SD], 1.27 [0.39]; median, 1.00) (Table). Interestingly, all MCC specimens positive for MCPyV were also positive for phospho-PKCε. Total PKCε, including active and inactive forms, was positive in all (11 of 11 [100%]) MCC specimens with a mean (SD) grading of 3.55 (0.52) (median, 4.00); MCPyV was positive in 8 of 11 cases of MCC (73%), with a mean (SD) grading of 2.64 (1.75) (median, 3.00).

Discussion | Merkel cell carcinoma is an older-age-onset-preference tumor1 that was initially attributed to be of spontaneous origin from Merkel cells. However, it is now well-recognized that MCPyV is a trigger in most cases of MCC.2 About 80% of MCC presents with MCPyV positivity,3 although this percentage varies among different geographic areas.4

In this report, we used immunohistochemistry (Figure) for phospho-PKCε to investigate the presence of these molecules in MCC specimens. There was an extremely strong correlation between PKCε activation and MCPyV positivity. Our hypothesis is that treatments targeting PKCε in MCC may augment current therapies for MCC caused by MCPyV, including immunotherapy, chemotherapy, and radiation therapy.

Adilson Costa, MD, PhD
Jamie Mackelfresh, MD
Linda Gilbert, PhD
Michael Y. Bonner, BA
Jack L. Arbiser, MD, PhD

Author Affiliations: Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia (Costa, Mackelfresh, Gilbert, Bonner, Arbiser); Atlanta Veterans Administration Medical Center, Atlanta, Georgia (Costa, Gilbert, Bonner, Arbiser), Department of Pathology, Emory University School of Medicine, Atlanta, Georgia (Mackelfresh).

Corresponding Author: Jack L. Arbiser, MD, PhD, Department of Dermatology, Emory University School of Medicine, WMB 5309, 1639 Pierce Dr, Atlanta, GA 30322 (arbiser@emory.edu).

Accepted for Publication: March 23, 2017.

Published Online: May 31, 2017. doi:10.1001/jamadermatol.2017.1296

Author Contributions: Drs Arbiser and Costa had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Arbiser.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Costa, Bonner, Arbiser.

Critical revision of the manuscript for important intellectual content: All authors.

Obtained funding: Arbiser.

Administrative, technical, or material support: Bonner, Arbiser.

Study supervision: Costa, Mackelfresh, Arbiser.

Conflict of Interest Disclosures: None reported.

Funding/Sponsor: This study was supported in part by Emory Skin Disease Research Core Center Grants from the National Institutes of Health (grants RO1 AR47950 and P30 AR42687), a Veterans Administration Hospital Merit Award (No. 11018002326–01A1), as well as funds from the Margolis Foundation, Rabinowitch-Davis Foundation for Melanoma Research, and the Betty Minsk Foundation for Melanoma Research.

Role of the Funder/Sponsor: The funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We are indebted to Gustavo Facchini, BS, MSc, PhD, Kosmoscience Group, Campinas, SP, Brazil, who kindly provided statistics support on this project. Dr Facchini was not compensated for his contributions.


An Anchoring-Based Intervention to Increase Patient Willingness to Use Injectable Medication in Psoriasis

Biological therapies are highly effective in treating immune-mediated diseases, such as psoriasis,5 but patients are often fearful of injections. The fear of injection is inherently subjective and may be easily modified.

Anchoring is the tendency for humans to make judgments relative to the first piece of information presented.2,3 Patients who have never taken an injection may subjectively compare taking an injection to not taking one. This comparison can be a frightening hurdle to starting an injectable medication. In a similar fashion, a $500 product (the anchor) marked down to $100 seems like a much better deal than if the product were simply offered at $100.

Since psychological anchors can be arbitrary, resetting the anchor may be a potential approach to help patients overcome fear of injection. We assessed whether patients offered a once-monthly injectable option would be more amenable to the injection if initially anchored to a once-daily injectable.

Methods | Participants received a study fact sheet letter detailing the study and informed consent was obtained verbally and assumed based on patient completion of the questionnaire. They were not compensated for participating. Written consent was waived by the institutional review board at the Wake Forest School of Medicine, which approved the study, because the research presented no more than minimal risk of harm to participants and involved no procedures for which consent is normally required outside of the research context. One hundred patients with a clinical diagnosis of psoriasis, aged 18 years or older, and not prescribed an injectable medication were recruited and randomized to either completing a control or intervention questionnaire. The 50 participants in the intervention group were first asked an anchoring question assessing their willingness to start a once-daily injectable medication and then subsequently queried on their willingness to start a once-monthly injection for their psoriasis (a scale of 1-10 with 1 being “not willing” and 10 being “very willing”). The 50 participants in the control group were only asked their willingness to start a once-monthly injection. Scores were treated as ordinal data and evaluated using the Mann-Whitney U test.

Results | There were no significant differences between the groups’ baseline characteristics (Table). Participants an-