Activation of Protein Kinase C epsilon in Merkel Cell Polyomavirus-Induced Merkel Cell Carcinoma

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Merkel cell carcinoma (MCC) is a rare neuroendocrine tumor of the skin1 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 PKCε (PKCe) (EPR1482[2], ab124806; Abcam Inc), phospho–PKCε (phosphorylated S729, ab63387; Abcam Inc), or MCPyV large T-antigen (CM2B4, sc-136172; Santa Cruz Biotechnology Inc) diluted 1:100 with Dako EnVision and Dual Link System–HRP (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>
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<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2/F/80s</td>
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<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>
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<td>3</td>
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</tr>
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<td>7/M/90s</td>
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<td>10/F/60s</td>
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<td>4</td>
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</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, Hematoxylin-eosin, and (B) positive staining for activated protein kinase Cε.

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Phospho-PKCε seems to be positive in 8 of 11 MCC specimens (73%) (grading 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, 3.00). 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 tumor\(^1\) 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.\(^4\) About 80% of MCC presents with MCPyV positivity,\(^5\) although this percentage varies among different geographic areas.\(^6\)

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.

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.

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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.

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