

Rb is Hyperphosphorylated in Most Merkel Cell Carcinomas



Elaine Cham, MD
Pathology – 2650 Ridge Avenue
Evanston, IL 60201
echam@northshore.org

EM Cham¹, TA Victor¹, R Orr¹, M Morgan¹, KW Lannert¹, SM Share², TC Pereira², WB Laskin³, CD Sturgis⁴

¹Pathology, NorthShore University HealthSystem, Evanston, IL; ²Pathology, Allegheny General Hospital, Pittsburgh, PA; ³Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL; ⁴Pathology, CellNetix, Seattle, WA



BACKGROUND

Merkel cell carcinoma (MCC) is an aggressive skin tumor with epithelial and neuroendocrine differentiation. MCC oncogenesis is not well understood, but may involve polyomavirus (PyV) mediated dysregulation of the retinoblastoma (Rb) pathway. Rb controls cell cycle progression. During cell transition from G1 to S phase, Rb is converted from a hypo- to hyperphosphorylated state. Previously, we showed that p16, an upstream regulator of Rb, is overexpressed in nearly all MCCs. The aim of our study is to characterize the expression pattern of Rb in MCCs.

DESIGN

45 specimens from 34 patients obtained from 3 institutions' archives (NSUHS 1992-2007, NMH 2000-2006, AGH 1994-2005), included 28 primary, 11 metastatic, 5 recurrent, and 1 MCC without available info. There were 27 (60%) males, 17 (38%) females, and 1 patient without available info. Mean age was 60 years (44-94 years). A tissue microarray with 3 to 5 cores (0.6 mm) of each MCC was made. Anti-human phospho-Rb (pRb, conc. polyclonal antibody, 1:500, Cell Signaling Technology) IHC was done with antigen retrieval (decloaking chamber, Biocare Medical). Results for pRb staining were interpreted by EMC and scored on a semiquantitative 4-tiered scale.

RESULTS

Nuclear and/or cytoplasmic reactivity for pRb was seen in 83% (37 of 45) of the MCCs. 76% (22 of 29) of primary, 91% (10 of 11) of metastatic, and 80% (4 of 5) of recurrent MCCs showed nuclear and/or cytoplasmic staining in varying intensities. Of the 37 MCCs positive for pRb, 22, 11, and 4 had nuclear only, nuclear + cytoplasmic, and cytoplasmic only staining, respectively. Of the 33 MCCs with nuclear staining, 12 and 21 showed strong and weak intensity, respectively. Of the 15 MCCs with cytoplasmic staining, all showed weak intensity.

CONCLUSIONS

Our data show most MCCs express pRb in the nucleus and/or cytoplasm. This Rb hyperphosphorylation explains the high proliferation rate seen in many MCCs and why p16 is overexpressed in most MCCs. The pRb expression patterns varied in cellular location, reactivity, and intensity, suggesting multiple mechanisms may be responsible for the Rb dysregulation. PyV transforms cells through deregulation of tumor suppressors such as Rb. Our results are consistent with studies showing that human PyV may play a role in MCC oncogenesis.

Introduction

Merkel cell carcinoma (MCC) is an aggressive primary cutaneous neoplasm showing both epithelial and neuroendocrine differentiation. The molecular events leading to MCC are not well understood, however, there is increasing evidence that it involves Merkel cell polyomavirus (MCPyV)-mediated dysregulation of the retinoblastoma (Rb) pathway. Rb is a tumor suppressor gene that is functionally inactivated in numerous pediatric and adult cancers. The Rb pathway has not been well-characterized in MCC. Rb is a central regulator of cell cycle progression and, during cellular transition from G1 to S phase, it is inactivated when it is converted from a hypo- to hyperphosphorylated state.

MCPyV-mediated oncogenesis may be analogous to that of high-risk human papillomavirus, which causes cervical cancer when viral oncoproteins (E6 and E7) disrupt the cell cycle by binding to Rb. Viral inactivation of Rb causes p16, an upstream regulator of Rb, to be overexpressed in cervical carcinomas. Previously, we have shown that p16 is also overexpressed in nearly all MCCs.

The purpose of this study is to characterize the expression pattern and functionality of Rb in MCCs.

Methods

45 specimens from 34 patients obtained from 3 institutions' archives (NSUHS 1992-2007, NMH 2000-2006, AGH 1994-2005), included 28 primary, 11 metastatic, 5 recurrent, and 1 MCC without available information.

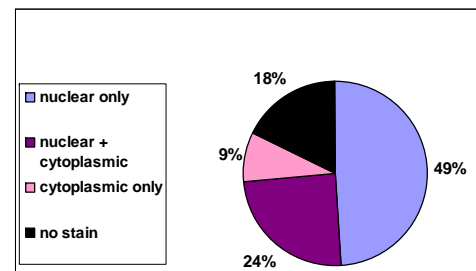
There were 27 (60%) males, 17 (38%) females, and 1 patient without available info. Mean age was 60 years (44-94 years).

A tissue microarray with 3 to 5 cores (0.6 mm) of each MCC was made. Anti-human phospho-Rb (pRb at Ser608, concentrated polyclonal antibody, 1:500, Cell Signaling Technology) IHC was done with antigen retrieval (decloaking chamber, Biocare Medical). Results for anti-human phospho-Rb at Ser608 (pRbSer608) staining were interpreted by EMC and scored on a semiquantitative 4-tiered scale.

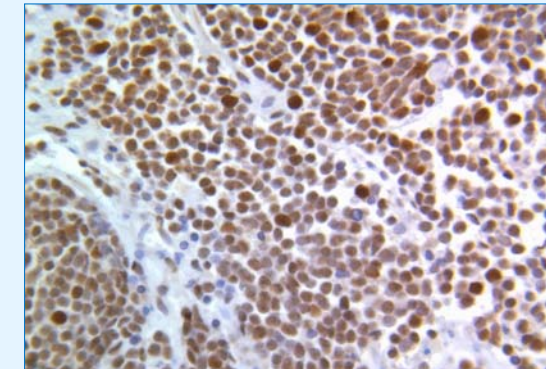
Results

Nuclear and/or cytoplasmic reactivity for pRbSer608 was seen in 83% (37 of 45) of the MCCs.

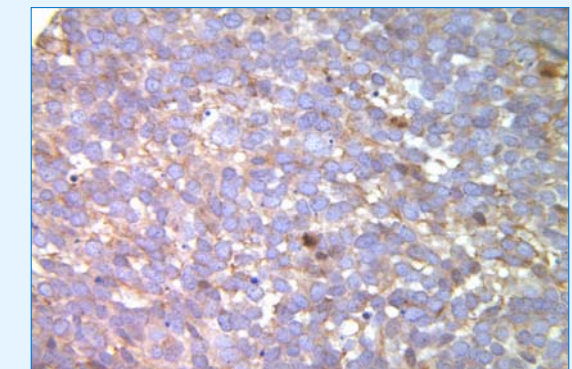
76% (22 of 29) of primary, 91% (10 of 11) of metastatic, and 80% (4 of 5) of recurrent MCCs showed nuclear and/or cytoplasmic staining in varying intensities. Of the 37 MCCs positive for pRbSer608, 22, 11, and 4 had nuclear only, nuclear + cytoplasmic, and cytoplasmic only staining, respectively. Of the 33 MCCs with nuclear staining, 12 and 21 showed strong and weak intensity, respectively. Of the 15 MCCs with cytoplasmic staining, all showed weak intensity.



Summary of pRbSer608 staining patterns in MCC



pRbSer608 nuclear staining – 40x



pRbSer608 cytoplasmic staining – 40x

Discussion

Studies from independent groups have corroborated that MCPyV plays a role in MCC oncogenesis, but exactly how this virus causes malignant transformation has not yet been elucidated. Our data demonstrate that Rb is phosphorylated at Ser608 in the nucleus and/or cytoplasm in the majority of MCCs. This suggests that most MCCs express Rb, functionally inactivated through phosphorylation. Our findings may explain the high proliferation rate seen in many MCCs and why p16 is also overexpressed.

In our study, pRbSer608 expression patterns varied in cellular location, reactivity, and intensity, suggesting multiple pathways are responsible for the Rb dysregulation. In the subset of MCCs that did not stain for pRbSer608, it may be that: a) there is homozygous deletion of Rb gene and/or mutation(s) involving the locus for the Rb Ser608 phosphorylation site; b) Rb is present but not phosphorylated or phosphorylated at a site other than Ser608; c) the Ser608 site is already bound, perhaps by an activating viral protein.

There are multiple mechanisms in which the MCPyV could directly or indirectly disrupt the Rb pathway. Polyomaviruses have a multifunctional T antigen oncoprotein capable of transforming cells through deregulation of cell cycle regulatory proteins such as Rb. T antigen genes have been found to be commonly expressed in all MCCs. MCPyV large T antigen retains the conserved domains common to polyomaviruses such as pocket Rb binding LXCXE and DnaJ. In addition to cancer initiation by the T-antigen oncoprotein, MCPyV have been shown to be capable of insertional mutagenesis, as it integrates into the MCC genome in a clonal pattern.

Additional studies are needed to delineate the events that lead to MCC carcinogenesis. A better understanding of the interplay between MCPyV and Rb will potentially provide the knowledge needed to design novel targeted therapies for MCC. Members of viral oncogenic pathways that inactivate Rb are potentially such therapeutic targets.

References

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