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Whole exome sequencing of Merkel cell carcinoma demonstrates conserved retinoblastoma pathway dysregulation

Merkel cell carcinoma is a rare aggressive neuroendocrine malignancy of the skin that is associated with infection by Merkel cell polyomavirus. Viral integration into the human genome and subsequent expression of the large T antigen is thought to cause cell cycle dysregulation via binding and inactivation of the retinoblastoma tumor suppressor protein and is a key step in the development of Merkel cell carcinoma. However, a small subset of Merkel cell tumors are polyomavirus-negative, suggesting that viral integration may not be required for tumorigenesis. While polyomavirus-negative Merkel cell carcinoma was traditionally thought to have a worse prognosis, recent studies have shown similar outcomes and significant overlap in the clinical and morphologic features of polyomavirus-positive and polyomavirus-negative Merkel cell carcinoma. Therefore, it was hypothesized that similar molecular pathways may be involved in the pathogenesis of both forms of the carcinoma. To probe this hypothesis, the authors examined eight cases of primary Merkel cell carcinoma of the skin. Five of the cases were polyomavirus-positive and three were polyomavirus-negative, as determined by standard polymerase chain reaction and real-time PCR methods. Total genomic DNA was extracted from paraffin-fixed tissue, and whole exome sequencing was performed. The sequences were compared to the human reference genome to find single nucleotide polymorphisms, small and large insertions and deletions, translocations, and copy number variations. Mutation recurrence among all eight cases was used to determine the genes implicated in Merkel cell carcinogenesis. While there were no gene variants specific to polyomavirus-positive Merkel cell carcinoma, it was discovered that all three cases of polyomavirus-negative Merkel cell carcinoma had nonsense truncating mutations in the RB1 gene that encodes the retinoblastoma protein. These three truncating mutations were seen in exons 3, 6, and 18, but all were nonsense mutations predicted to be deleterious. No nonsense truncating mutations were seen in the RB1 gene in the polyomavirus-positive cases, although a copy number variation was seen in one case and a deletion mutation in another. To further validate these mutations, immunohistochemistry for the retinoblastoma protein was performed. The five tumors with RB1 mutations had negative staining, but strong nuclear staining was observed in the tumors without RB1 mutations. Interestingly, immunohistochemical staining for the activated phosphorylated retinoblastoma protein was negative in all eight cases, signifying that dysfunction of the retinoblastoma pathway is conserved in all cases of Merkel cell carcinoma. Overall, this study suggests that inactivation of the retinoblastoma protein and dysfunction of its tumor suppressor pathway is a critical step in Merkel cell pathogenesis. In polyomavirus-positive cases, expression of the large T antigen that binds to and perturbs the retinoblastoma protein is sufficient for this action. However, somatic mutation of the RB1 gene is an alternative mechanism of inactivating the retinoblastoma pathway. In either case, dysfunction of the retinoblastoma pathway seems to play a vital role in the pathogenesis of Merkel cell carcinoma. Therefore, targeted molecular therapy against components of the retinoblastoma pathway, especially those downstream of retinoblastoma, may be effective in polyomavirus-positive and polyomavirus-negative Merkel cell carcinoma.

Cimino PJ, Robirds DH, Tripp SR, et al. Retinoblastoma gene mutations detected by whole exome sequencing of Merkel cell carcinoma. *Mod Pathol*. 2014;27:1073-1087.

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