Cell Cycle Markers in Merkel Cell Carcinoma

Clinical Research

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Merkel Cell Carcinomas: Expression of S-Phase Kinase-Associated Protein 2 (Skp2), p27, and Proliferation Markers

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Abstract

Merkel cell carcinomas are rare and aggressive tumors about which the expression of cell cycle regulatory proteins are not well known. We evaluated the clinicopathologic features of Merkel cell carcinomas and examined the expression of the cell cycle regulatory markers p27 and S-phase kinase-associated protein 2 (Skp2) and the proliferation markers Ki-67 and DNA topoisomerase II alpha (topo II alpha) in a group of these tumors. Thirty-nine cases of Merkel cell carcinoma were studied, 19 from the Mayo Clinic, Rochester, MN, and 20 from the University of Torino, Torino, Italy. Although the University of Torino patients tended to be slightly older at time of surgery compared to the Mayo Clinic patients, no clinical, pathologic, or immunohistochemical feature was statistically significantly different between the two groups. Of the 39 patients, 20 were male and 19 were female. The age at surgery averaged 72 yr. Formalin-fixed paraffin-embedded archival tissues from the 39 Merkel cell carcinomas were analyzed by immunohistochemistry for p27, Skp2, Ki-67, and topo II alpha with the avidin–biotin peroxidase system. The distribution of immunoreactivity was analyzed by quantifying the percentage of positive nuclei, which was expressed as the labeling index. There was a statistically significant inverse relationship between p27 and Skp2 ($p = 0.005$). Most tumors with increased levels of Skp2 were associated with reduced p27, and tumors with high levels of p27 expression were associated with reduced levels of Skp2. These results suggest that Skp2 regulates p27 expression in Merkel cell carcinomas. Tumors showing increased Skp2 expression were not always correlated with increased proliferation as evaluated by Ki-67 and topo II alpha, suggesting that Skp2 may be involved in Merkel cell tumorigenesis, but that other factors may also influence cell proliferation in these tumors.

Key Words: Merkel cell carcinoma; cell cycle; proliferation markers; p27; S-phase kinase-associated protein 2; Skp2; Ki-67; DNA topoisomerase II alpha.

Introduction

Merkel cell carcinomas are uncommon aggressive tumors. The immunohistochemical profile for these tumors is characteristic and diagnostically distinctive. Merkel cell carcinomas stain for chromogranin A and synaptophysin [1–3] and show juxtanuclear staining for cytokeratin 20, Cam5.2, and other low-molecular-weight cytokeratins [4,5], but they do not stain for thyroid transcription factor-1 [6–8]. This immunophenotype is diagnostically characteristic of Merkel cell carcinomas and assists in their distinction from metastatic small cell carcinoma of pulmonary origin [6–8]. Although the diagnostic immunophenotype of Merkel cell carcinoma is known, the expression and biologic
significance of cell cycle regulatory proteins in Merkel cell carcinomas is not known.

Recent studies have shown that p27kip1 (p27) expression has diagnostic and prognostic significance in a variety of human cancers, including endocrine, colon, breast, and lung tumors [9–18]. The p27 gene codes for a cyclin-dependent kinase inhibitor protein that helps to regulate the transition from the G1 to the S phase of the cell cycle by binding and inhibiting cell cyclin-dependent kinases [19,20]. Expression of p27 protein decreases as cells progress from the G1 to the S phase of the cell cycle [21]. The importance of this gene product in tumor growth is supported by the observation that p27 deficient mice show increased growth and a syndrome of multiorgan hyperplasia with tumorigenesis, suggesting p27 may be a tumor suppressor gene [22,23]. However, the p27 gene is rarely mutated in human tumors [24,25].

Regulation of p27 has been shown to occur post-transcriptionally, by inhibitory cytokines such as transforming growth factor-beta [26] and by degradation through the ubiquitin-proteosome pathway [27,28].

The S-phase kinase-associated protein 2 (Skp2) has been implicated in the ubiquitin-mediated degradation of the p27 [29–32]. Skp2 is an F-box protein thought to function as a receptor component of a ubiquitin ligase complex that targets p27 for degradation [29–33]. Expression of Skp2 has been shown to correlate inversely with p27 expression in non-Hodgkin’s lymphomas [34], oral squamous cell carcinomas [35], and colorectal carcinomas [36]. The relationship between levels of p27 and its specific ubiquitin ligase subunit Skp2 has not been evaluated in Merkel cell carcinomas.

Ki-67 antigen is a marker of proliferation that is expressed in all phases of the cell cycle except G0 [37]. Topoisomerase II alpha (topo II alpha) is also a proliferation marker [38–42] and functions to prevent nondisjunction and chromosome breakage in mitosis [41,42]. Topo II alpha has been utilized to assess proliferative activity in a variety of neoplasms [39,40]. The relationship between Skp2 and proliferation markers in Merkel cell carcinomas is not known.

The objectives of this study are to evaluate the expression of p27, Skp2, Ki-67, and topo II alpha in Merkel cell carcinomas. We observed an inverse relationship between p27 and Skp2 expression in Merkel cell carcinomas suggesting that Skp2 is involved in Merkel cell tumorigenesis.

Materials and Methods

Cases and Tissues

Formalin-fixed parafilm-embedded archival tissues from 39 randomly selected patients who underwent surgery at the Mayo Clinic, Rochester, MN or the University of Torino, Torino, Italy between 1984 and 1999 were used. Hematoxylin and eosin–stained (H&E) sections were reviewed independently by three of the authors (L.A.E., M.P., R.V.L.) for verification of diagnoses (Fig. 1). Clinical history and follow-up information including mean age, gender, tumor size, and outcome were obtained by chart review (Table 1). Permission for the study was obtained from the Mayo Clinic Institutional Review Board.

Immunohistochemical Analysis

Formalin-fixed, paraffin-embedded tissue sections were cut at 5 μm and treated with 0.1 mol/L citrate, pH 6.0, in an 800-W microwave oven for 15 min for antigen retrieval before immunostaining.