Lack of Wnt5A Expression in Merkel Cell Carcinoma

Merkel cell cancer (MCC) is a rare skin cancer of neuroendocrine origin, the onset of which can be induced by chronic sun exposure.1 The molecular mechanisms underlying the development and progression of MCC are unclear. Recently, it has been shown that the Merkel cell polyomavirus (MCV) is causally linked to MCC. The DNA of MCV can be detected in approximately 80% of MCCs and has been demonstrated to be monoclonally integrated into the genome of the tumor cells that express the MCV large T antigen.2

The noncanonical Wnt signaling pathway is characterized by proteins such as Wnt5A, which is important in the progression of skin cancers such as melanoma.3 Wnt5A has also been shown to antagonize β-catenin signaling, a member of the canonical Wnt signaling pathway. No mutations in key members of the canonical Wnt pathway (eg, CTNNB1 or APC) have been identified in MCC, and nuclear accumulation of β-catenin has been shown in a small percentage of MCC.4 The somewhat unusual lack of β-catenin activation in such an aggressive tumor, coupled with the fact that Wnt5A expression is high in other skin tumors, led us to hypothesize that perhaps Wnt5A is highly expressed in MCC and suppresses β-catenin.

Methods. We analyzed MCV-positive and MCV-negative MCC cell lines for Wnt5A expression. We used 2 well-established MCC lines, Mkl-1 (MCV+/−) and UISO (MCV−), and 2 additional cell lines (BroLi and MaTi), generated from patients in our clinic with MCC. BroLi has been shown by real-time polymerase chain reaction (PCR) to carry MCV DNA, while MaTi cells are virus negative. All cells were grown in RPMI 1640 medium (Sigma Chemical Co, St Louis, Missouri) supplemented with 10% fetal calf serum prior to lysis in radioimmunoprecipitation assay buffer. Western blot analysis for Wnt5A expression was performed as described previously.5

Results. Western analysis revealed no significant differences in Wnt5A expression among MCCs of differing metastatic or MCV status (Figure 1), and Wnt5A expression was very low in these cells. A metastatic melanoma cell line, M93-047, with a positive upper band for Wnt5A was used as the positive control. This upper band was absent in almost all MCC cell lines but registered faint positivity in the UISO cells.

To confirm these findings, we also stained a tissue microarray of 32 primary and 17 metastatic MCC samples.
Using real-time PCR analysis of tissue from tissue blocks that were subsequently cored to construct the tissue microarray, we found that MCV DNA was present in 44 of these tumors and absent in 5 (data not shown). Wnt5A staining was low or absent in virtually all samples analyzed (Figure 2A), and no difference between virus-positive and virus-negative samples was observed. Staining of a primary melanoma was used as a positive control for Wnt5A staining, where Wnt5A positive cells stained brown (Figure 2B).

Comment. These data suggest that Wnt5A is not involved in the onset or progression of MCC and support other data that indicate that Wnt signaling is inactive in this cancer despite its neuroendocrine origins.

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Combined Treatment With Rituximab and Anthracycline-Containing Chemotherapy for Primary Cutaneous Large B-Cell Lymphomas, Leg Type, in Elderly Patients

Primary cutaneous large B-cell lymphoma, leg type (PCLBCL,LT), mainly affects the elderly population. Nevertheless, to our knowledge, no specific studies are available on the outcomes in patients 80 years or older. Herein, we aim to evaluate the efficacy and tolerance of a modified R-CHOP regimen (rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone) in this particular population.

Methods. Four patients aged 81 to 96 years (mean age, 91 years) were treated and followed up for PCLBCL,LT. Their general condition in all cases was good prior to treatment, and pretherapeutic echocardiography showed no severe ventricular dysfunction, thus allowing anthracycline therapy. All patients had multiple lesions; the lesions were confined to 1 inferior limb in 3 patients and were disseminated in the fourth case. Whole-body computed tomographic scans showed extracutaneous involvement in only 1 patient, who had a laterotracheal lymphadenopathy. An osteomediullary biopsy was performed in 1 patient, and no medullar involvement was found.

All patients received a modified R-CHOP treatment with reduced doses of the following drugs: doxorubicin, 25 mg/m²; vincristine, 1 mg/m²; and cyclophosphamide, 400 mg/m². Rituximab and prednisone were given at standard doses (ie, 375 mg/m² and 40 mg/m² for 5 days, respectively). A prophylactic injection of pegfilgrastim was administered the day after chemotherapy. Cycles were administered every 3 or 4 weeks.

Results. All patients achieved a partial or complete remission. Three patients experienced severe infections requiring hospitalization. Two patients had congestive heart failure: one had decreased ventricular function and myocardial infarction, and the other experienced worsening of a preexisting chronic atrial fibrillation. None of our patients experienced severe cytopenias, mucitis, or relapse or neurologic toxic effects. In 2 cases, the treatment had to be discontinued, and these patients died of adverse effects. The other 2 completed 6 cycles of treatment and at last follow-up (15 and 21 months) remained alive and in complete remission. Clinical data and outcomes are summarized in the Table.

Comment. In the overall population, the outcome of PCLBCL,LT is improved by R-CHOP, which is now con-