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DEK expression in Merkel cell carcinoma and small cell carcinoma

Background: The chromatin architectural factor DEK maps to chromosome 6p and is frequently overexpressed in several neoplasms, including small cell lung carcinoma, where it is associated with poor prognosis, tumor initiation activity and chemoresistance. DEK expression has not been studied in cutaneous Merkel cell carcinoma.

Methods: We applied a DEK monoclonal antibody to 15 cases of Merkel cell carcinoma and 12 cases of small cell carcinoma. DEK nuclear immunoreactivity was scored based on percentage (0, negative; 1+, <25%; 2+, 25–50%; 3+, >50%) and intensity (weak, moderate or strong).

Results: All 15 Merkel cell carcinoma cases (100%) showed diffuse (3+) nuclear positivity (14 strong, 1 weak). Six of 12 small cell carcinoma cases (50%) showed diffuse (3+) and strong nuclear positivity, while one case exhibited focal (1+) weak nuclear positivity. The remaining five cases were negative for DEK expression.

Conclusions: Our results suggest that DEK may be involved in the pathogenesis of Merkel cell carcinoma and therefore may provide therapeutic implications for Merkel cell carcinomas. In addition, the difference in DEK expression between Merkel cell carcinoma and small cell carcinoma suggests possible separate tumorigenesis pathways for the two tumors.

Keywords: DEK, Merkel cell carcinoma, neuroendocrine carcinoma, small cell carcinoma

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DEK, a highly conserved nuclear factor, is an abundant and structurally unique constituent of metazoan chromatin and is highly expressed in proliferating cells.[1] *In vitro* studies have shown that DEK is a chromatin architectural factor and has effects on mRNA splicing, transcriptional control, DNA damage repair, differentiation, cell viability, cell to cell signaling and chemoresistance.[2–5] In the cell, these functions might be integrated by the profound impact of DEK on heterochromatin.[6] DEK also exhibits prooncogenic effects including inhibition of p53-mediated apoptosis, cooperation with viral oncogenes E6 and E7 to overcome senescence, and promotion of HRAS-driven keratinocytic transformation.[7,8]

DEK is encoded on chromosome 6p and was initially discovered as the target of a chromosomal translocation event t(6;9)(p23;q34) in a subset

of acute myeloid leukemias.[9] The t(6;9)(p23;q34) translocation results in an in-frame fusion between DEK and nucleosporin NUP214, which localizes to the nucleoplasm.[10] Expression of the DEK-NUP214 fusion correlates with activation of protein synthesis and phosphorylation of eIF4E, a translational initiation factor.[11] Subsequent studies have reported DEK overexpression independent of the t(6;9) translocation in a number of human malignancies, including retinoblastoma, glioblastoma, hepatocellular carcinoma, bladder carcinoma, colon cancer, gynecologic cancers, breast cancer, laryngeal cancer, melanoma, large cell neuroendocrine carcinoma and small cell lung carcinoma.[3,5,12–19] Indeed, DEK is a key factor in controlling the notorious chemoresistance of melanoma.[3]

Merkel cell carcinoma, referred to as small cell carcinoma of the skin, represents a rare, highly aggressive cutaneous neuroendocrine neoplasm with 33% mortality within 3 years of diagnosis.[20] In the United States, 1500 cases of Merkel cell carcinoma are diagnosed annually.[21] From 1986 to 2001 there has been a threefold increase in Merkel cell carcinoma cases, likely due to an expansion of the immunosuppressed population and elevated awareness.[22] Molecular studies show trisomy 6 as the most common chromosomal abnormality, present in almost 50% of tumors.[23]

Non-cutaneous small cell carcinoma, another highly aggressive neuroendocrine tumor, has a dismal 5-year survival of 5–10%. Small cell carcinoma most commonly occurs within the lung and accounts for approximately 15% of primary lung carcinomas.[24] It also rarely occurs in extrapulmonary sites, which typically carries a worse prognosis. Chromosomal gain of 6p22.3, the DEK locus, in small cell carcinoma is associated with a worse prognosis.[25]

Skin lesions of Merkel cell carcinoma and metastatic small cell carcinoma are usually indistinguishable by cytomorphology, necessitating a detailed clinical history and a panel of immunohistochemical stains. Typically, Merkel cell carcinoma is positive for CK20 and negative for thyroid transcription factor (TTF)-1, while small cell carcinoma reacts with TTF-1 but not with CK20.[26] However, there are exceptions to these staining patterns, requiring a systemic work-up to exclude metastatic small cell carcinoma to the skin or soft tissue. The overexpression of DEK in small cell carcinoma and large cell neuroendocrine carcinoma raises the question of whether DEK could be upregulated in Merkel cell carcinoma. In this study, we examine the expression of DEK in Merkel cell carcinoma as compared to small cell carcinoma.

Table 1. Summary of patient demographics for cases of Merkel cell carcinoma and small cell carcinoma

Diagnosis	n	Sex (M : F)	Age range (mean)
Merkel cell carcinoma	15	8 : 7	59–86 (74)
Primary	9	6 : 3	59–84 (78)
Metastatic	6	2 : 4	59–86 (78)
Small cell carcinoma	12	5 : 7	45–83 (59)
Primary	10	4 : 6	45–83 (59)
Metastatic	2	1 : 1	56–58 (57)

M, male; F, female.

Materials and methods

After University of Michigan Institutional Review Board approval, a tissue microarray (TMA) containing 15 well-characterized Merkel cell carcinomas from 12 patients in triplicate was previously constructed from archival formalin-fixed paraffin-embedded tissue (FFPET) identified through a retrospective search of the University of Michigan pathology database. Nine of 15 Merkel cell carcinoma cases were collected from primary cutaneous excisions or excisions of locally recurrent tumors. The remaining six specimens were collected from distant metastases or lymph node metastases. A retrospective search for archival FFPET blocks yield 12 cases of well-characterized small cell carcinoma with 10 pulmonary (8 lung, 1 bronchus and 1 mediastinum) and 2 extrapulmonary lesions (metastasis from lung to adrenal gland and paraspinal soft tissue, respectively). The original hematoxylin and eosin-stained sections, along with available immunohistochemical stains, were reviewed by two dermatopathologists (L.M. and R.P.) to confirm the diagnosis. Table 1 summarizes the patient demographics.

FFPET 5-μm tissue sections from the TMA and conventional paraffin blocks were deparaffinized and pretreated with citrate buffer at pH 6.0. After antigen retrieval sections were incubated with mouse monoclonal anti-DEK antibody (1 : 400 dilution; BD Transduction Laboratories, Franklin Lakes, NJ, USA) at room temperature for 30 min, followed by EnVision+System horseradish peroxidase-conjugated goat anti-mouse (Dako, Carpinteria, CA, USA) for 30 min. Sections were then treated with peroxidase substrate solution containing 0.01% hydrogen peroxide and 0.05% diaminobenzidine tetrahydrochloride (Dako) and counterstained with hematoxylin. Tonsil tissue served as positive control.

Nuclear staining was considered positive for DEK expression. The staining was scored and recorded based on percentage (0 = negative, 1 = <25%, 2 = 25–50%, 3 = >50%) and intensity (weak, moderate or strong) by two dermatopathologists

Table 2. Percentage and intensity of DEK positive tumor cells in cases of Merkel cell carcinoma and small cell carcinoma

	n	Negative (%)	<25% and weak (%)	≥25% and weak (%)	<25% and strong (%)	≥25% and strong (%)
Merkel cell carcinoma	15	0 (0)	0 (0)	1 (6.7)	0 (0)	14 (93)
Primary	9	0 (0)	0 (0)	0 (0)	0 (0)	9 (100)
Metastatic	6	0 (0)	0 (0)	1 (17)	0 (0)	5 (83)
Small cell carcinoma	12	5 (42)	1 (8.3)	0 (0)	0 (0)	6 (50)
Primary	10	5 (50)	1 (10)	0 (0)	0 (0)	4 (40)
Metastatic	2	0 (0)	0 (%)	0 (0)	0 (0)	2 (100)

(L.M. and R.P.). The staining patterns and intensities among three cores showed minimal variation.

Fisher's exact test was performed to assess the difference in DEK expression between Merkel cell carcinoma and small cell carcinoma using GraphPad Prism version 5.01 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com. A p-value <0.05 was considered statistically significant.

Results

In normal skin, as previously described, DEK was expressed with moderate intensity in basilar keratinocytes and dermal lymphocytes.[27] In normal lung tissue, DEK staining was restricted to infiltrating lymphocytes.[5]

The staining pattern of DEK in Merkel cell carcinoma and small cell carcinoma are summarized in Table 2. All 15 cases (100%) of Merkel cell carcinoma showed diffuse (3+) nuclear reactivity. Nearly all cases (14/15) showed strong staining (Fig. 1A), except that one case of metastatic Merkel cell carcinoma exhibited weak staining. In contrast, 7 of 12 (58%) small cell carcinoma cases showed positive DEK staining, which was significantly less than for Merkel cell carcinoma ($p < 0.05$). Among seven DEK-positive small cell carcinoma cases, six exhibited diffuse (3+) and strong nuclear reactivity (Fig. 1B) while one case had focal (1+) and weak staining. Both cases of metastatic small cell carcinoma exhibited diffuse and strong DEK staining. All five cases of DEK-negative small cell carcinomas (Fig. 1C) were pulmonary primary tumors with four from lung and one from mediastinum.

Discussion

DEK represents a highly conserved nuclear factor with function regarding chromatin and DNA damage repair. The overexpression of DEK has been reported in numerous human malignancies, including retinoblastoma, bladder carcinoma, colon cancer and melanoma. More recently, DEK expression was found in high grade neuroendocrine lung cancers, including small cell and large cell carcinomas, where elevated DEK levels were associated with a worse prognosis.[5] In this study, we examined the expression of DEK by immunohistochemistry in Merkel cell carcinoma in comparison with small cell carcinoma of the lung. To our knowledge, this is the first report to examine DEK expression in Merkel cell carcinoma.

In this study, we found an upregulation in DEK expression in nearly all (14/15) Merkel cell carcinomas and about half (7/12) of small cell carcinoma cases. Strong overexpression of DEK in our small cell carcinoma cohort is comparable to a previous study that showed strong overexpression of DEK in 44.3% (35/79) of high grade neuroendocrine lung carcinomas.[5] Although there is a significant difference in DEK expression in Merkel cell carcinoma and small cell carcinoma, the presence of DEK expression would be of limited diagnostic use in distinguishing the two entities since a strong and diffuse staining pattern may be seen in both entities. However, the difference in DEK staining patterns in Merkel cell carcinoma and small cell carcinoma suggests that the two tumors may follow different pathways in tumorigenesis. Similarly, Merkel cell carcinoma and small cell carcinoma vary in their p63 expression patterns. Small cell carcinoma is typically negative for p63.[28] In contrast, p63 overexpression was identified in 61% of lower stage Merkel cell carcinomas.[29] Interestingly, DEK overexpression stimulates p63 expression at the level of mRNA and protein synthesis. In addition, knockdown of DEK expression results in p63 repression in a keratinocyte cell line.[30] These findings suggest the difference in DEK expression between Merkel cell carcinoma and small cell carcinoma may be, in part, related to the difference in p63 expression noted between the two entities.

DEK expression is positively regulated by the high-risk human papilloma virus (HPV) E7 oncogene via a decrease in the expression of retinoblastoma protein, a tumor suppressor.[7] Early DEK studies in HPV infections showed that decreased retinoblastoma protein results in overexpression of DEK.[7] Recently, a 5387 base pair polyomavirus genome was discovered in 43–100% Merkel cell carcinoma

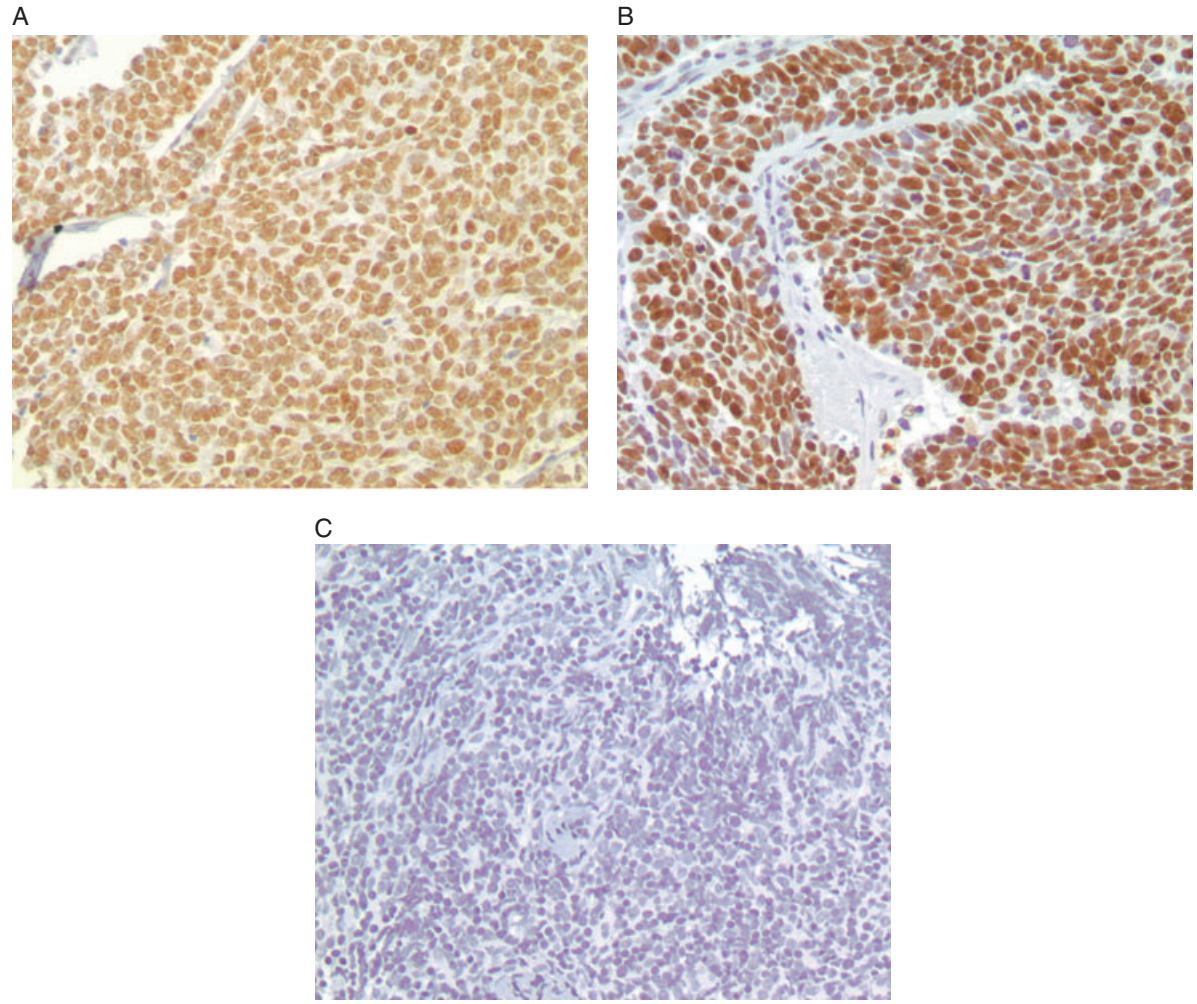

Fig. 1. Representative DEK immunohistochemistry in Merkel cell carcinoma (A) and small cell carcinoma (B) demonstrating diffuse, strong nuclear reactivity and in small cell carcinoma demonstrating an absence of reactivity (C).

tumors.[31–33] The virus, named MCPyV because of its association with Merkel cell carcinoma, integrates into different sites of the cellular genome in different Merkel cell carcinoma tumors and cell lines. The MCPyV-encoded large T antigen binds retinoblastoma protein, which plays a vital role in Merkel cell carcinoma tumorigenesis.[34] We suspect that the overexpression of DEK in Merkel cell carcinoma may be a result of MCPyV genome integration and the downregulation of retinoblastoma protein. In contrast, MCPyV is identified in only 0–39% of small cell carcinomas, which is significantly lower than Merkel cell carcinomas.[35–37]

We found that the overexpression of DEK not only in nine primary/locally recurrent tumors but also six metastatic tumors, indicating that the level of DEK expression probably plays no significant role in the prognosis of Merkel cell carcinoma. A future study with a larger cohort and detailed clinical follow-up data is required to confirm our initial findings. Patients with high-grade neuroendocrine lung cancers expressing high levels of DEK have a significantly shorter survival than those expressing low levels of DEK (18 versus 55% overall survival after 11 years).[5] In our study, 100% (2/2) of metastatic small cell carcinomas strongly expressed DEK, whereas 44% (4/9) of primary/recurrent tumors showed positivity. Our findings suggest that DEK may correlate with a more aggressive phenotype. However, a relationship between DEK expression and clinical outcome cannot be concluded from this study because of the limited time course and information for follow up.

The purported role of DEK in tumorigenesis in a number of human malignancies combined with its high incidence of expression in Merkel cell carcinoma makes DEK an appealing therapeutic target. Silencing of DEK expression in small cell lung carcinoma cell clones transplanted in immunodeficient mice resulted in near abrogation of tumor formation compared to parent clones.[5] In melanoma cell lines, inhibition of DEK expression

led, among other effects, to cell cycle arrest.[3] In addition, reduction of DEK expression resulted in increased sensitivity to chemotherapeutic agents in small cell lung carcinoma and melanoma cell lines.[3,5] The ubiquitous DEK expression in Merkel cell carcinoma makes it an appealing candidate for DEK suppression therapy.

In summary, DEK is diffusely and strongly expressed in nearly all Merkel cell carcinomas but only in half of small cell carcinomas. The difference in DEK staining patterns between these two neuroendocrine tumors suggests the possibility of distinct oncogenic pathways. The frequent DEK overexpression in Merkel cell carcinoma makes it an appealing therapeutic target. Our study provides a basis for a future large cohort study to investigate the therapeutic utility of DEK in Merkel cell carcinomas.

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