Reduced Expression of RECK Protein May Help Differentiate Cutaneous Malignant Melanoma from Melanocytic Nevus

LEI ZHANG[1], QIANG XIE[2], SHI-JUN SHAN[3], MEBRATU KETEMA TABOR[2] and JACQUELINE M. JUNKINS-HOPKINS[2]

[1]*Dermatology Hospital of Jiangxi Province, Nanchang, P.R. China;*
[2]*Ackerman Academy of Dermatopathology, New York, NY, U.S.A.;*
[3]*Department of Dermatology, Tianjin Medical University General Hospital, Tianjin, P.R. China*

Abstract. *Background: Differentiating between vertical growth cutaneous malignant melanoma (CMM) and predominantly intradermal benign melanocytic nevus (BMN) can be extremely challenging. Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is an endogenous inhibitor of many proteins that promote tumor invasion and progression. We investigated the difference in expression of RECK between CMM and BMN to determine whether RECK could assist in their differentiation. Materials and Methods: The expression of RECK in skin biopsies of 39 cases, including 20 cases of CMM and 19 cases of BMN, was evaluated by immunohistochemistry. Results: Two out of 20 cases (10%) of CMM were positive for RECK, and all (100%) 19 cases of BMN exhibited positivity at different levels. In 7/8 cases of CMM adjacent to benign nevus cells, malignant melanoma cells did not express RECK, but benign nevus cells stained positively. Conclusion: These findings suggest that immunohistochemical staining for RECK could be useful in the differential diagnosis between CMM and BMN.*

The incidence of cutaneous malignant melanoma has steadily increased over the past 30 years (1). A total of 1,658,370 new cancer cases and 589,430 cancer-related deaths are projected to occur in the United States in 2015(2). Histopathological analysis remains the gold standard for the pathological diagnosis of melanoma, and incorporates many features, such as cytological atypia, asymmetry, poor circumscription, failure of maturation, and mitotic activity. In most cases, a diagnosis can be made with confidence based on these features, however, in some cases, making a differential diagnosis between vertical growth cutaneous malignant melanoma (CMM) and predominantly intradermal benign melanocytic nevus (BMN) can be extremely challenging. Immunohistochemical stains, such as S100-protein, human melanoma black 45 (HMB45), Melanoma Antigen Recognized by T-cells 1(MART-1), Ki-67 and Micropthalmia transcription factor (MITF) (3-6), have provided some additional diagnostic information to help differentiate benign from malignant melanocytic neoplasms, yet an accurate diagnosis still may not be established with certainty. Additionally, thickness, which is an important prognostic factor (7), may be difficult to determine in CMM adjacent to benign nevus cells due to the difficulties in distinguishing melanoma components from benign nevus cells. Hence, there is still considerable disagreement among pathologists on the diagnosis and reporting of melanoma (8-12).

Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) is a membrane-anchored glycoprotein, first discovered as a tumor suppressor by screening a human fibroblast cDNA library for genes giving rise to reversion-inducing clones (13). RECK can suppress tumor invasion and metastasis by negatively regulating matrix metalloproteinases (MMPs) involved in carcinogenesis, such as MMP2, MMP9 and MMP14 (14-17), which are essential for extracellular matrix remodeling (18, 19). RECK is expressed in various normal human tissues, being involved in neurogenesis (20) and hematopoietic stem and progenitor cell retention and migration (21), but has not been detected in several cancer types. Consequently, RECK is considered a good prognostic indicator in some malignancies (22, 23).

Considering that CMM is invasive but BMN is not, we investigated the difference in expression of RECK in these two conditions by immunohistochemistry.

Correspondence to: Jacqueline M. Junkins-Hopkins, Ackerman Academy of Dermatopathology, 145 East 32nd Street 10th Floor, New York, NY 10016, U.S.A. Tel: +1 8005536621, Fax: + 1 2128898268, e-mail: jakki22@gmail.com.

Key Words: Melanoma, nevus, reversion-inducing cysteine-rich protein with Kazal motifs, immunohistochemical stain, RECK.

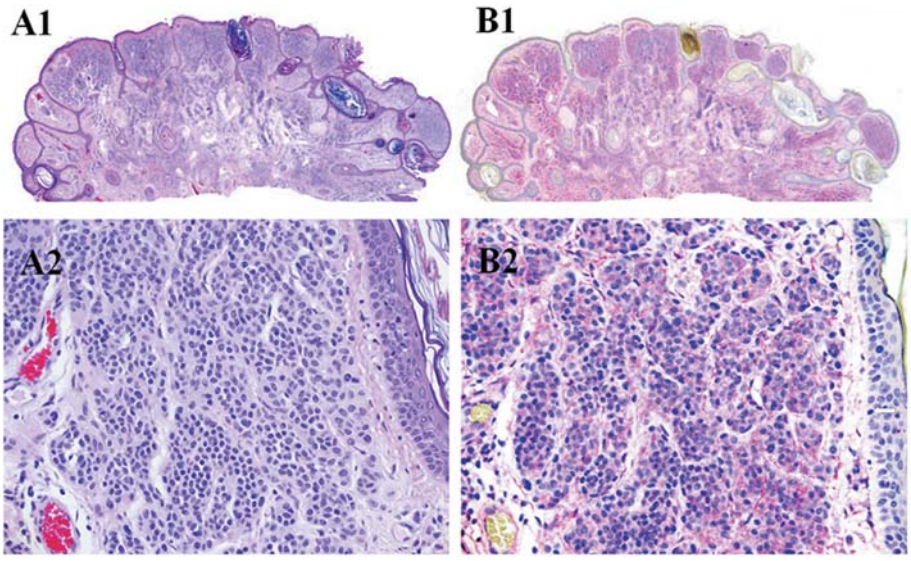

Figure 1. *Staining for reversion-inducing cysteine-rich protein with Kazal motifs (RECK) in benign intradermal melanocytic nevus lesion. A: Hematoxylin-eosin sections show bland melanocytic cells in the dermis (original magnifications A1: ×4, A2: ×20). B: Immunohistochemical staining for RECK (red) is strongly positive in the cytoplasm and membrane of nevus cells, and negative in normal melanocytic cells (B2, arrow) (original magnifications B1: ×4 , B2: ×20).*

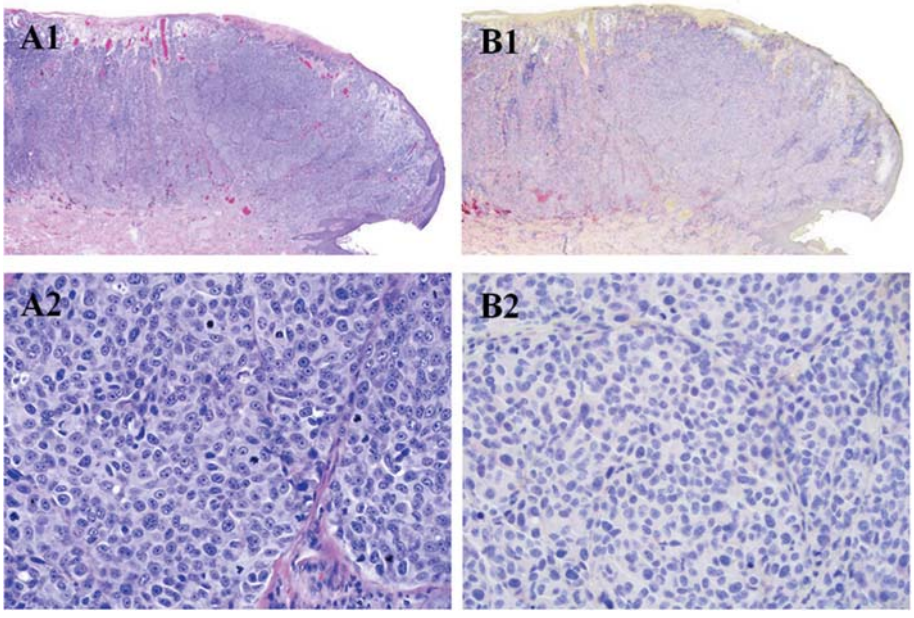

Figure 2. *Staining for reversion-inducing cysteine-rich protein with Kazal motifs (RECK) in cutaneous malignant melanoma lesions. A: Hematoxylin-eosin sections show atypical melanocytic cells in the dermis with ulceration and many mitotic figures (original magnifications A1: ×4, A2: ×40). B: Immunohistochemical staining for RECK (red) is negative (original magnifications B1: ×4 , B2: ×40).*

Materials and Methods

Patients and specimens. Thirty-two patients with CMM or BMN, who had undergone histological examination at the Ackerman Academy of Dermatopathology, New York, NY, USA, from January 2012 to October 2013, were included in this study. In 20 patients with CMM [mean age (±SD)=60.2±20.9 years; male=14, female=6], there were 19 cases of vertical growth phase melanoma, and one case of nodular melanoma. In these CMMs, there were eight cases of vertical growth phase melanoma adjacent to benign nevus cells. The

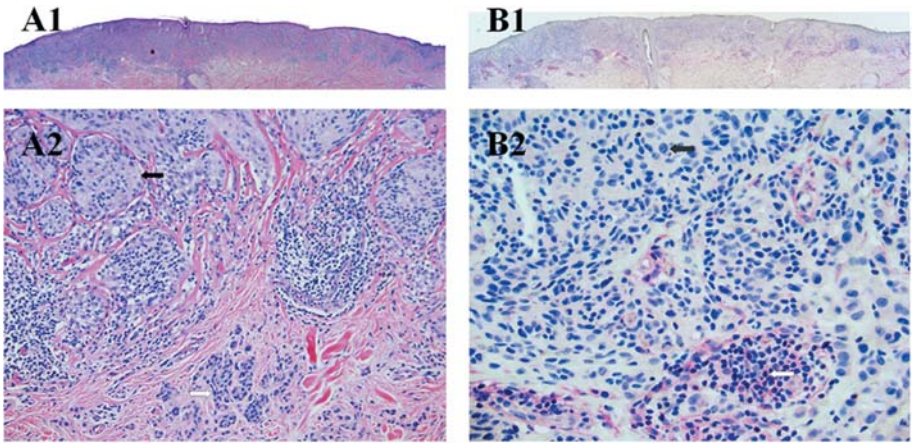

Figure 3. *Staining for reversion-inducing cysteine-rich protein with Kazal motifs (RECK) in cutaneous malignant melanoma adjacent to nevus lesion. A: Hematoxylin-eosin sections show atypical melanocytic cell proliferation (A2, black arrow), single or in nests in the epidermis and dermis with bland melanocytic nevus cells (A2, white arrow) at the bottom of the lesion (original magnifications A1: ×2.5, A2: ×20). B: Immunohistochemical staining for RECK (red) is negative in malignant melanoma cells (B2, black arrow), but strongly positive in benign nevus cells (B2, white arrow) (original magnifications B1: ×2.5, A2: ×20).*

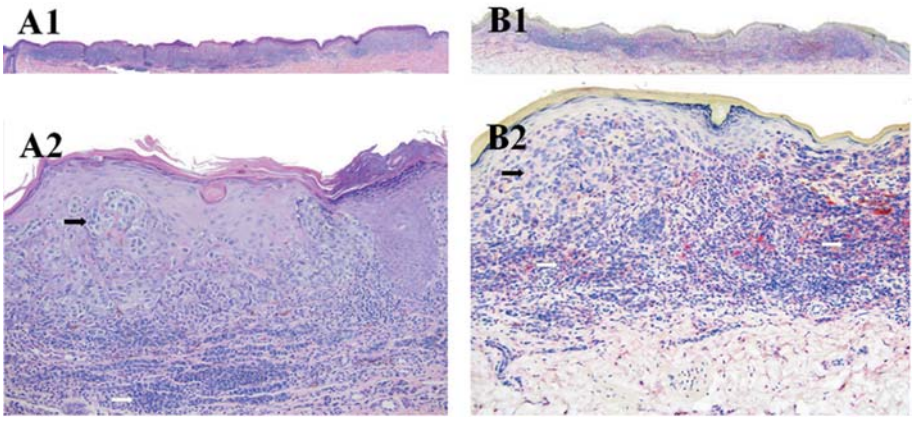

Figure 4. *Staining for reversion-inducing cysteine-rich protein with Kazal motifs (RECK) in cutaneous malignant melanoma adjacent to nevus. A: Hematoxylin-eosin sections show atypical melanocytic cell proliferation (A2, black arrow), single or in nests in the epidermis and dermis with bland melanocytic nevus cells (A2, white arrow) at the bottom of the lesion (original magnifications A1: ×2.5, A2: ×20). B: Immunohistochemical staining for RECK (red) is weakly positive in malignant melanoma cells (B2, black arrow), but more strongly positive in bland nevus cells (B2, white arrow) (original magnifications B1: ×2.5, B2: ×20).*

following clinicopathological features were collected: sex, age, lesion location, subtype, RECK expression, Breslow thickness, mitosis, and presence of ulceration. Twelve patients (age=38.8±17.6 years; male=2, female=10) with 19 cases of BMN were included. All the specimens were formalin-fixed paraffin-embedded tissue sections of the skin lesions from these patients.

Immunohistochemical assays. Tissue sections of 5 μm thickness were cut then heated at 80˚C for 30 min. Slides were stained using Leica BOND-III (Leica Microsystems, Buffalo Grove, Illinois, USA). Retrieval was performed using Epitope retrieval solution 2 (Leica Microsystems, Buffalo Grove, Illinois, USA.) for 20 min. The sections were incubated in rabbit antibody to human RECK (diluted 1:200 (D8C7); rabbit IgG monoclonal antibody; Cell Signaling Technology, Danvers, Massachusetts, USA) at 37˚C for 30 min. Detection was carried out using Bond Polymer Refine Red Detection Kit (Leica Microsystems Inc.) in accordance with the manufacturer's protocol. Slides were stained using Hematoxylin and dehydrated in graded alcohol. Human brain tissue was stained as a positive control, and negative controls were prepared by omitting the primary antibody.

All slides were examined, recorded and analyzed by three investigators independently. The staining intensity of immunohistochemical assays was graded as: –, no staining; +,

Table I. *Clinicopathological data of the patients and reversion-inducing cysteine-rich protein with Kazal motifs (RECK) expression in vertical growth phase cutaneous malignant melanoma.*

No./gender/age (years)	Lesion location	Histologic type	BT (mm)	Ulceration	Mitotic rate (/mm^2)	Metastatic	RECK expression
1/M/33	Left torso	MM	2.9	+	0	–	MC:–
2/M/46	Right anterior thigh	MM	1.1	–	0	–	MC:++
3/F/35	Right jawline	MM	1.1	–	2	–	MC:–
4/F/36	Left lateral breast	MM	0.3	–	0	–	MC:–
5/M/66	Lower abdomen	MM	3.2	+	2	–	MC:–
6/M/54	Upper back	MM	29	–	0	–	MC:–
7/M/89	Left posterior shoulder	MM	3.0	+	0	–	MC:–
8/M/94	Left lateral foot	MM	4.6	+	15	+	MC:–
9/F/85	Right anterior thigh	MM	7	+	17	+	MC:–
10/M/85	Left lower leg	MM	1.1	–	0	–	MC:–
11/M/78	Right thigh	MM	3.1	+	20	+	MC:–
12/F/51	Right breast	MN (nodular)	0.9		1	–	MC:–
13/F/36	Left leg	MMN	0.48	–	0	–	MC:+ NC:++
14/M/75	Right arm	MMN	0.64	–	0	–	MC:– NC:+++
15/M/80	Right shoulder	MMN	1.3	+	2	–	MC:– NC:+++
16/M/32	Left anterior scalp	MMN	0.58	–	0	–	MC:– NC:+++
17/M/45	Left shoulder	MMN	0.32	–	0	–	MC:– NC:++
18/F/66	Right leg	MMN	0.53	–	0	–	MC:– NC:++
19/M/48	Right central back	MMN	0.2	–	0	–	MC:– NC:++
20/M/69	Right ear	MMN	1.7	–	1	–	MC:– NC:++

MMN, Vertical growth phase melanoma adjacent to benign nevus; MC, malignant melanoma cell; NC, benign nevus cell; BT, Breslow thickness.

faint staining; ++, clearly positive staining; +++, strong positive staining. Multiple lesions from one patient were dealt with statistically as multiple cases.

Fisher's exact test was used to statistically analyze the data; a value of $p<0.05$ was regarded as indicating statistical significance.

Results

The clinicopathological data of the cases are summarized in Tables I, II and III. In 19 cases of BMN, benign nevus cells all expressed RECK in the cytoplasm and membrane at different levels, but normal melanocytes in the marginal skin of BMN did not express RECK (Figure 1). In CMM, the expression of RECK was not detected in 18 cases (18/20) of CMM (Figure 2); and in other 2/20 cases of CMM, (Figure 4 and 5) diffuse fine granular cytoplasmic staining was detected in malignant melanoma cells and melanophages, with no staining on the membrane, in contrast to the membranous granular pattern noted in BMN. In 7/8 cases of CMM adjacent to benign nevus cells, malignant melanoma cells stained negatively, but the benign nevus cells stained positively in the cytoplasm and membrane (Figure 3); benign nevus cells in 1/8 cases stained positively in the cytoplasm and membrane, and malignant melanoma cells stained weakly positive in the cytoplasm (Figure 4). A statistically significant difference was observed between the expression of RECK in CMM and BMN ($p<0.01$). There was no significant difference in RECK expression intensity observed by CMM histopathological type, Breslow thickness, the presence or absence of ulceration, mitotic rate and metastasis.

Negative immunohistochemical staining for RECK had a sensitivity of 100% (95% confidence interval=78-100%) and a specificity of 90.48% (95% confidence interval=68-98%) as a diagnostic marker of CMM in the present study.

Discussion

In the study, all benign melanocytic nevi expressed RECK in the cytoplasm and membrane at different levels, but most CMM did not. In most cases of the CMM adjacent to benign nevus cells, the malignant melanoma cells stained

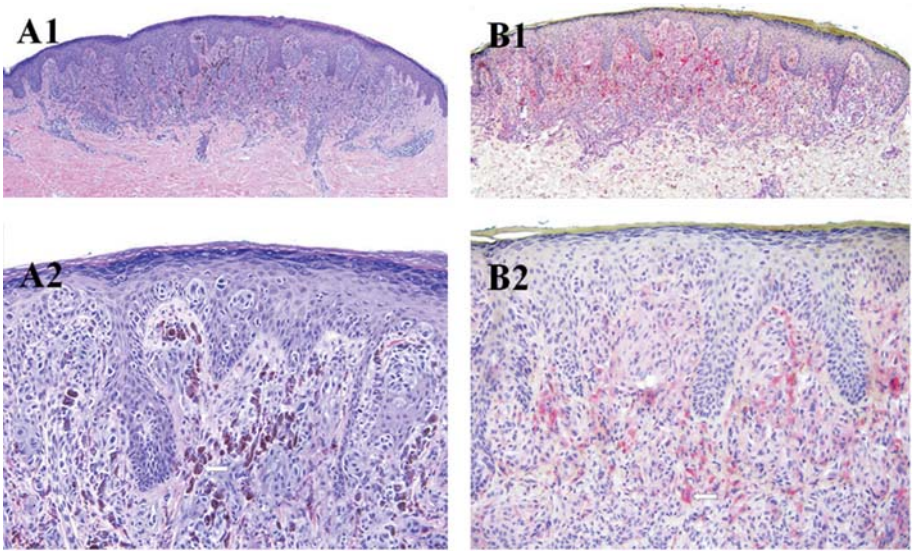

Figure 5. *Staining for reversion-inducing cysteine-rich protein with Kazal motifs (RECK) in cutaneous malignant melanoma. A: Hematoxylin-eosin sections show atypical melanocytic cell proliferation in the epidermis and dermis. The melanocytes contain abundant melanin and are admixed with numerous melanophages (A2, white arrow) (original magnifications A1: ×4, A2: ×20). B: Immunohistochemical staining for RECK (red) shows diffuse fine granular cytoplasmic staining detected in malignant melanoma cells and melanophages, with no staining on the membrane (B2, white arrow) (original magnifications B1: ×4, B2: ×20).*

Table II. *Clinicopathological data of the patients and Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) expression in intradermal benign melanocytic nevi.*

No./gender/age (years)	Lesion location	RECK expression
1/F/20	Left oral commissure	++
2/M/23	Right upper back	++
3/F/38	Left lower chest	++
	Right lower chest	+++
4/M/74	Left lateral chin	+
5/F/31	Left mid back	+++
6/F/22	Right side of bridge of nose	+
7/F/63	Left arm/wrist	+++
8/F/51	Right cheek	+++
	Right glabella	++
	Left nasal sill	+++
	Left cheek	++
9/F/54	Forehead	++
	Left chin	+
10/F/34	Left chest	+++
	Mid chest	++
11/F/26	Posterior neck	+++
12/F/30	Right posterior ear	+++

Table III. *Reversion-inducing cysteine-rich protein with Kazal motifs (RECK) expression in different patients.*

	CMM	BMN
Total cases	20	19
Cases with RECK expression	2*	19

CMM, Vertical growth phase cutaneous malignant melanoma; BMN, intradermal benign melanocytic nevus; *p=0.0001.

negatively, but benign nevus cells stained positively. These findings suggest that RECK could play a role as a diagnostic marker differentiating CMM from BMN. Moreover, in CMM adjacent to benign nevus cells, assessment of RECK expression may provide assistance in measuring the thickness of CMM.

In one of the two cases of CMM that stained positively for RECK, it was notable that the tumor was composed of melanocytes with abundant melanin and admixed with prominent melanophages. We considered that the pattern of staining, being finely granular in the cytoplasm, in contrast to the pattern noted in BMN, implied that the stain was not specific for RECK. It is possible that this may be related, in part, to the melanin pigment; however, a similar staining pattern was noted in the other positive melanoma that had less melanin. The explanation for the positive staining in these two melanomas remains elusive. Close inspection of the pattern of staining of RECK is important in order to differentiate the staining pattern of benign nevus from that of melanoma. The role for RECK in differentiating CMM from BMN needs to be explored on a larger scale.

We were able to show a potential role in evaluating melanoma in the context of nevoid presentation. Since the preparation of our article, we have become aware of a report preliminarily released online that also documents the correlation between RECK and melanoma. In this report, the expression of RECK was analyzed in two publicly available melanoma microarray datasets and in a panel of human melanoma cell lines, RECK was shown to be down-regulated (26). Further support for RECK as a diagnostic marker in melanoma is manifested by the finding that expression of activated MMP2, which is inhibited by RECK, correlates with increased malignancy in a xenograft model of melanoma (27).

A relationship between diminished RECK expression and malignancy has also been reported in renal and urothelial cell proliferative neoplasms. RECK was strongly reduced in these malignant proliferations, but highly expressed in benign counterparts (22, 23). In lymphoid tumors, RECK expression levels were significantly reduced, and this down-regulation of RECK indicated a more aggressive disease, and a poor prognosis (24). Decreased RECK expression was also found to be an independent prognostic factor of poor survival (25). *RECK* gene polymorphisms might be a risk factor increasing hepatocellular carcinoma susceptibility and distant metastasis (28). Investigations of the promoter methylation of *RECK* revealed possible relationships with many types of cancers (29, 30), but not with cutaneous melanoma (31). Overexpression of RECK in malignant tumor cells was associated with reduced invasiveness (32). These investigations support the notion that RECK may play an important role in progression of many cancer types.

In summary, we provided data to support a potential role for assessment of RECK expression in differentiating melanoma from benign predominantly dermal melanocytic nevus. Our study has limitations, including small size, and exclusion of subtypes of nevi, including spitz, combined, atypical, and desmoplastic melanocytic neoplasms. These subtypes and evaluation of melanoma *in situ*, borderline melanocytic tumors, and metastatic disease should also be included in future investigations of RECK. Furthermore, because RECK has been found to be a target of malignant transformation in lung, breast, prostatic, and colorectal cancer (33-37), our findings highlight a potential role for assessment of RECK expression in the management of melanoma.

Conflicts of Interest

The Authors have no conflict of interest to declare.

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