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Expression of activated Akt in benign nevi, Spitz nevi and melanomas

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Abstract

Background—Activated Akt expression (p-Akt) is reportedly increased in many melanomas as compared with benign nevi. The purpose of this study was to evaluate and compare p-Akt immunohistological staining in benign nevi, Spitz nevi and primary melanomas.

Methods—Immunostaining for phosphorylated Akt was performed in 41 melanocytic lesions previously classified as benign intradermal nevus (14 lesions), Spitz nevus (9 lesions) or melanoma (18 lesions). Lesions were graded for intensity of p-Akt staining by two independent observers (0, no staining; 1, slightly positive; 2, moderately positive; 3, highly positive). Scores were averaged, and statistical analyses were performed.

Results—Benign nevi showed less staining (mean score 1.18) compared with Spitz nevi (mean score 2.11) and melanomas (mean score 2.19). This difference was statistically significant between benign nevi and melanomas ($p = 0.0047$) and benign nevi and Spitz nevi ($p = 0.0271$). No statistical difference was detected in staining between Spitz nevi and melanomas ($p = 0.8309$).

Conclusions—Activated Akt expression is increased in Spitz nevi and melanomas as compared with benign intradermal nevi, but is unlikely to prove useful in differentiating between the former.

Melanocytic lesions are frequently difficult to distinguish histologically, and a consensus diagnosis may not be possible even among pathologists with expertise in this discipline.¹ In particular, differentiating between Spitz nevus and melanoma using light microscopy often poses a diagnostic challenge. A better understanding of the molecular events leading to the progression of benign melanocytes to melanoma may lead to the discovery of useful discriminating immunohistochemical markers.

Constitutive nuclear factor (NF)- κ B(p65/RelA) and activated Akt expression have been described in melanoma.^{2,3} Akt/PKB is a serine/threonine kinase, which is a core component of the phosphatidylinositol 3-kinase signaling pathway.⁴ Akt promotes the transcription of many genes involved in cell cycle progression, cell proliferation and survival.⁵ Akt may

suppress apoptosis by activating NF- κ B.⁶ Activated Akt also phosphorylates and inactivates several pro-apoptotic proteins, including BAD(Bcl-XL/Bcl-2-Associated Death Promoter)⁷,⁸ and caspase-9.⁹ Activation of Akt has been described in many human cancers, including ovarian, breast, pancreatic, stomach and gliomas.^{4,10–13}

A previous study showed no significant immunostaining for activated Akt in 10 benign melanocytic nevi, while 8 of 12 metastatic melanomas showed moderate to highly positive immunostaining.² Dai et al. evaluated 292 pigmented lesions and noted increased p-Akt expression by immunohistochemical staining in dysplastic nevi, primary melanomas and melanoma metastases as compared with benign nevi. Increasing p-Akt expression was inversely correlated with patient overall and disease-free survival, and it was a poor prognostic factor for patients with melanomas less than 1.5 mm in thickness.¹⁴ The purpose of our study was to evaluate and compare activated Akt expression in benign intradermal nevi, Spitz nevi and primary cutaneous melanomas using immunohistochemical staining. To our knowledge, Akt immunostaining has not been previously examined in Spitz nevi.

Materials and methods

Following IRB approval (no. 040061), 14 benign intradermal melanocytic nevi, 9 Spitz nevi and 18 primary cutaneous melanomas were randomly selected from the archives of the Vanderbilt University Dermatopathology service. The histological diagnosis was confirmed by a board-certified dermatopathologist (ASB).

Five-micron sections of formalin-fixed, paraffin-embedded tissues were placed on charged slides and dried overnight at 50°C. Paraffin was removed from the slides, and the tissue sections were rehydrated. The slides were placed in heated Target Retrieval Solution (DakoCytomation, Carpinteria, CA) and allowed to cool for 20 min at room temperature. A solution of 0.03% hydrogen peroxide was applied to each slide to neutralize endogenous peroxidase activity, followed by an application of casein-based protein block (DakoCytomation) for non-specific staining blocking. The sections were incubated overnight with rabbit anti-phospho-Akt (Cell Signaling Technology no. 9277) diluted 1:50 in Antibody Diluent (DakoCytomation). Sections without primary antibody served as negative controls. The Dako Envision + System/HRP (DakoCytomation) and Vector NovaRed (Vector Laboratories, Burlingame, CA) produced specific, visible staining. The slides were lightly counterstained with Mayer's hematoxylin, dehydrated and cover slipped.

Immunostaining was independently graded by two observers (JBR and SMK) blinded to the histological diagnosis: 0, no staining; 1, slightly positive; 2, moderately positive and 3, highly positive. Scores were based on a global assessment, combining intensity of staining and percentage of immunoreactive cells. The graders' scores were averaged. Analyses of study results focused on estimating the association between the activated Akt expression and different types of melanocytic lesions, i.e. benign nevi, Spitz nevi and melanomas. The tests of hypotheses concerning the associations between the mean expression levels of p-Akt and three study groups were carried out using the general linear model method with the Bonferroni multiple comparisons adjustment. All tests of significance were two sided, and differences were considered statistically significant when p value was <0.05. All data were expressed as means $\pm$ SD. SAS version 9.1.3 was used for all analyses.

Results

The quantitative results are given in Table 1. Intradermal nevi showed a mean staining intensity of 1.18 as compared with a mean staining intensity of 2.11 in Spitz nevi and 2.19 in melanomas. As shown in Figs. 1–3, Spitz nevi and melanomas displayed increased activated Akt

immunoreactivity compared with benign nevi. Lesions showed a mixture of cytoplasmic and nuclear staining.

Statistical analysis showed a significant difference in staining between benign intradermal nevi and melanomas ($p = 0.0047$) and benign intradermal nevi and Spitz nevi ($p = 0.0271$). There was no difference in staining intensity between Spitz nevi and melanomas ($p = 0.8309$).

Discussion

Currently, Spitz nevi are differentiated from melanomas by evaluating a constellation of histological features.¹⁵ Nevertheless, even lesions uniformly judged to have benign microscopic features may ultimately show aggressive biological behavior.¹⁶ Lesions that fail to meet the histologic criteria for melanoma but with features such as size greater than 10 mm, involvement of the subcutaneous fat, asymmetry, ulceration, poor circumscription, pagetoid melanocytosis, confluence of melanocytes, lack of maturation and zonation and absence of Kamino bodies are often characterized as atypical Spitz tumors, reflecting their uncertain biologic potential.¹⁷

Immunohistochemical markers that differentiate between Spitz nevi and melanomas would be useful diagnostically, and research regarding the divergent molecular pathways between these two entities may elucidate appropriate molecular targets in the future. Numerous markers, including Bcl-2, c-fos, c-kit, c-myc, cyclin D1, HMB45, hTERT (human telomerase reverse transcriptase), MART-1 (Melanoma Antigen Recognized by T-cells 1), p16, p27, proliferating cell nuclear antigen and survivin, failed to serve as reliable discriminators in previous investigations.^{18–29} Expression of activated Akt in Spitz nevi has not been previously studied to our knowledge. With similar levels of p-Akt expression noted in Spitz nevi and melanomas, staining for this protein does not appear to be useful in discriminating between these two entities. Activated Akt expression in Spitz nevi may account for the rapid rate of proliferation often seen clinically.

Our results are in concordance with those from other studies which reported increased p-Akt expression in melanomas as compared with benign nevi.^{2,15,30} Certain melanoma cell lines have been shown to express increased levels of activated Akt.² The heightened levels of activated Akt expression within some melanomas suggests that this signaling molecule might prove clinically useful for targeted antimelanoma therapy in the future.³¹ The main pitfall of our study is the small sample size.

In conclusion, Spitz nevi exhibit significantly activated Akt immunostaining when compared with benign intradermal nevi. Our results suggest that p-Akt is not a useful marker for discriminating between Spitz nevus and melanoma.

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Fig. 1.
Representative p-Akt staining results in an intradermal nevus. Intensity of staining in this lesion was graded as 0 (no staining) by both observers.

Fig. 2.

Representative p-Akt staining results in a Spitz nevus. A) Intensity of staining was graded as 3 (high-intensity staining) by both observers. B). Nuclear and cytoplasmic staining are evident on higher power.

Fig. 3. Representative p-Akt staining results in a melanoma. A) Intensity of staining was graded as 3 (high-intensity staining) by both observers. B) Nuclear and cytoplasmic staining are evident on higher power.

Table 1

Results of p-Akt immunohistochemical staining

	Number of cases	Average score (SMK)	Average score (JR)	Combined average
Intradermal nevi	14	1.28	1.07	1.18
Spitz nevi	9	2.11	2.11	2.11
Melanomas	18	2.17	2.22	2.19