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Expression of polycomb group protein EZH2 in nevi and melanoma

Background: Enhancer of zeste homolog 2 (EZH2), a polycomb group protein that regulates the cell cycle, has recently been implicated in the progression of several human cancers. We sought to determine the pattern of EZH2 expression in benign and malignant melanocytic tumors to see if EZH2 might play a role in melanoma pathogenesis and progression.
Methods: We identified and reviewed 11 compound nevi, 13 dysplastic nevi, 13 Spitz nevi, 9 *in situ* melanomas, 10 non-metastatic invasive melanomas and 19 melanomas metastatic to lymph nodes from the University of Michigan pathology archives. Sections immunostained with anti-EZH2 antibody were scored independently and blindly for staining intensity on a scale of 1–4 by three dermatopathologists. Results were analyzed and compared statistically.
Results: We observed an incremental increase in EZH2 expression from benign nevi to melanoma: scores of 1.18 and 1.08 for ordinary and dysplastic nevi, 1.7 and 1.78 for Spitz nevi and *in situ* melanoma, and 1.9 and 3.0 for invasive and metastatic melanoma, respectively. EZH2 expression for metastatic melanoma was significantly higher compared with invasive and *in situ* melanoma and benign nevi ($p \leq 0.01$).
Conclusions: EZH2 protein levels increase incrementally from benign nevi to melanoma, which suggests that EZH2 may play a role in the pathogenesis and progression of melanoma.

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The *Drosophila* protein homologue enhancer of zeste homolog 2 (EZH2) is one of the polycomb group of proteins involved in proliferation and the regulation of cell cycle progression.[1] Specifically, EZH2 is a histone M3 methyltransferase controlled by the E2F transcription factors that regulate the G_2 to mitosis transition[2] through nucleosome modification, chromatin remodeling and interaction with other transcription factors. Published data indicate that disruption of EZH2 expression in senescent fibroblasts retards cell proliferation and induces cell cycle arrest at the G_2 to mitosis transition,[3] while overexpression of EZH2 in cultured mouse embryonic fibroblasts shortens the G_1 phase of the cell cycle and leads to accumulation of cells in the S phase.[4] Dysregulation of these functions may lead to cancer or progression of cancer in humans.

EZH2 expression has recently been linked to the progression of many human cancers.[5–10] It was initially found to be associated with aggressive behavior of Hodgkin lymphoma.[5] Subsequently, it has been shown that EZH2 expression is also associated with aggressive behavior of prostate cancer,[6] breast cancer,[7–9] hepatocellular carcinoma[9] and urothelial carcinoma.[10] Recently, EZH2 was also found to be an independent poor prognostic marker in endometrial cancer and associated with features of aggressive cutaneous melanoma.[11]

Because EZH2 expression has been associated with progression in several cancers and little data exist regarding EZH2 expression in benign and malignant melanocytic tumors, we undertook the current study in order to evaluate levels of EZH2 protein expression in a spectrum of cutaneous

melanocytic lesions from benign nevi to metastatic melanoma.

Materials and methods

Case selection

This study was approved by the Institutional Review Board at the University of Michigan Health System. The study group consisted of a spectrum of cutaneous melanocytic lesions, including ordinary compound nevi (11), dysplastic (atypical, Clark's) nevi (13), *in situ* melanomas (9), invasive non-metastatic melanomas (10) and Spitz nevi (13) as well as melanomas metastatic to lymph nodes (19). In all 10 cases of invasive non-metastatic melanomas (4.0 mm mean Breslow depth, range of 1.46–11 mm), sentinel lymph node biopsies were negative and the patients had no evidence of recurrent or metastatic disease. These cases were sequentially identified by a SNOMED free text computer search of the pathology archives in the years between 1999 and 2001 at the University of Michigan Medical Center. Hematoxylin and eosin-stained sections were reviewed to confirm the above diagnoses.

Immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded 4-μm-thick tissue sections using standard biotin-avidin complex technique with a mouse monoclonal antibody against EZH2 (BD Biosciences, San Jose, CA, USA) at a 1:25 dilution.

Detectable nuclear staining was required to consider a case as positive for EZH2 expression as previously reported.[5–7] EZH2 protein expression intensity (Fig. 1 A–C) was scored as 1, negative; 2, low; 3, moderate; or 4, high following previously published guidelines.[6,7] Negative controls (no primary antibody) and positive internal controls (nuclei of keratinocytes or germinal center B cells) were used to validate the immunohistochemical staining procedure. EZH2 expression was evaluated blindly and independently by three dermatopathologists (D. R. F., L. M. and L. D. S.).

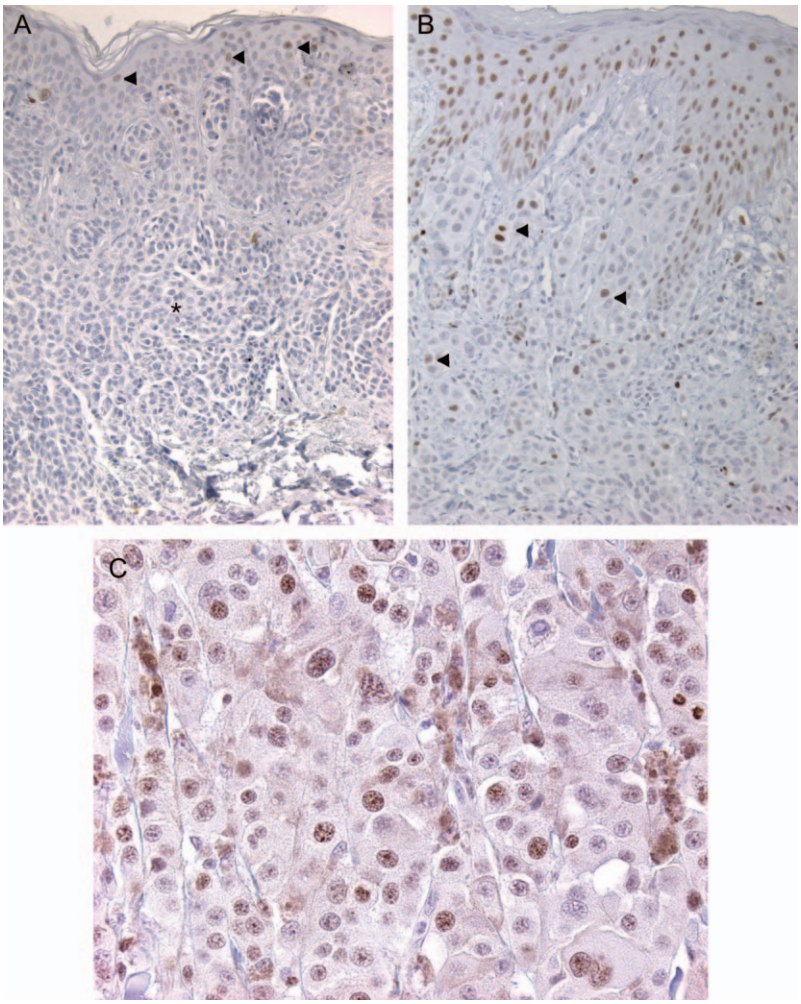

Fig. 1. A) Negative staining for enhancer of zeste homolog 2 (EZH2) (score = 1) in compound nevus (asterisk) with positively staining keratinocyte nuclei as internal control (arrowhead). B) Low-level expression of EZH2 (score = 2) in nuclei of Spitz nevus (arrowhead) with positively staining keratinocyte nuclei as internal control. C) High-level expression of EZH2 (score = 4) in nuclei of metastatic melanoma. Cytoplasmic melanin is also present.

Statistical analysis

The intensity of EZH2 immunohistochemical staining for the lesions was compared using the Mann-Whitney *U*-test (StatsDirect; StatsDirect Ltd, Cheshire, UK). A p-value of < 0.05 was considered statistically significant.

Results

There was good concordance among the three pathologists scoring EZH2 staining in immunoperoxidase preparations, with a complete concordance rate of 92%; five of the six discordant results differed by one value only. EZH2 expression was observed primarily in the nucleus as previously reported.[5–7] The mean scores are presented in Table 1. With the exception of one case, which had moderate and focal staining intensity for EZH2, all ordinary compound nevi were negative for EZH2 expression (Fig. 1A). Except for one case with low-level staining, all dysplastic nevi were negative for EZH2 expression and showed no significant difference in EZH2 expression compared with ordinary compound nevi (Table 2). Although *in situ* melanomas showed a trend toward higher EZH2 expression compared with ordinary compound nevi and dysplastic nevi (1.78 vs. 1.18 and 1.08, respectively), this did not achieve statistical significance. Interestingly, most Spitz nevi expressed EZH2 (Fig. 1B) and showed mean staining intensity similar to *in situ* melanomas (1.7 vs. 1.78). The differences between Spitz nevi and ordinary compound nevi as well as dyplastic nevi were only marginally significant ($p = 0.03$ and 0.02, respectively).

Table 1. Summary of EZH2 protein expression in a spectrum of cutaneous melanocytic lesions and metastatic melanomas

Diagnosis	N	Mean score
Compound nevus	11	1.18
Dysplastic nevus	13	1.08
Spitz nevus	13	1.7
Melanoma *in situ*	9	1.78
Invasive melanoma	10	1.9
Metastatic melanoma	19	3.0

In contrast, significantly increased EZH2 expression was seen in invasive melanoma with respect to ordinary compound nevi and dysplastic nevi, but not *in situ* melanoma. Invasive melanomas had a mean staining intensity of 1.9 compared with 1.18 and 1.08 for ordinary compound nevi and dysplastic nevi, respectively. These differences were statistically significant ($p = 0.02$ and < 0.01). In this group of invasive melanomas, there was no significant difference in EZH2 expression between tumors less than 2.5 mm in Breslow depth ($n = 6$) and tumors greater than 2.5 mm in Breslow depth ($n = 4$) ($p = 0.4$).

Metastatic melanomas showed the strongest EZH2 protein staining intensity (Fig. 1C). When melanomas were metastatic, the mean staining intensity was 3.0. This was significantly greater than ordinary compound nevi, Spitz nevi, dysplastic nevi (all $p \leq 0.01$) and *in situ* melanomas ($p \leq 0.01$), and remained so when compared with invasive melanomas ($p = 0.01$).

Discussion

In the present study, we found expression of EZH2 to be generally absent in ordinary compound nevi and dysplastic nevi, consistent with their generally dormant, non-replicating state. On the other hand, there was a trend for melanoma *in situ* to exhibit higher staining intensity with respect to ordinary compound nevi and dysplastic nevi, although this did not reach statistical significance. Importantly, invasive melanomas and melanomas metastatic to lymph nodes showed greater expression of EZH2 with respect to ordinary compound nevi and dysplastic nevi. Moreover, metastatic melanoma expressed EZH2 at significantly higher intensity compared with invasive melanomas. With respect to ordinary compound nevi, Spitz nevi expressed EZH2 to a higher degree but this was marginally significant and not to the degree of metastatic melanoma. Taken together, these findings suggest that EZH2 protein levels increase with malignant transformation of melanocytes, and that EZH2 may be involved in the metastatic process during melanoma progression.

Table 2. Summary of p-values comparing EZH2 immunohistochemical staining scores of melanocytic lesions

	CN	DN	SN	MIS	Inv Mel	Met Mel
CN	—	0.94	0.03	0.09	0.02	< 0.01
DN	0.94	—	0.02	0.05	< 0.01	< 0.01
SN	0.03	0.02	—	0.97	0.69	< 0.01
MIS	0.09	0.05	0.97	—	0.72	< 0.01
Inv Mel	0.02	< 0.01	0.69	0.72	—	0.01
Met Mel	< 0.01	< 0.01	< 0.01	< 0.01	0.01	—

CN, compound nevus; DN, dysplastic nevus; SN, Spitz nevus; MIS, melanoma *in situ*; Inv Mel, non-metastatic invasive melanoma; Met Mel, metastatic melanoma.

The precise role that EZH2 plays in melanoma tumor progression and the metastatic process is not known. However, Kleer et al.[7] has provided experimental evidence that EZH2 overexpression plays a role in neoplastic transformation. Using a breast cancer cell line, they demonstrated that overexpression of EZH2 promotes hallmark features of malignancy including anchorage-independent cell growth and invasion. Furthermore, in breast cancer patients, overexpression of EZH2 was associated with decreased disease-free survival and overall survival.

The only other study, to our knowledge, focusing on EZH2 expression in cutaneous melanoma was a very recent publication by Bachmann et al.[11] They evaluated 58 metastatic melanomas and found a 43% increase in median EZH2 expression compared with primary cutaneous melanomas. Our results are consistent with these findings. In their study, among 202 nodular melanomas, 5-year survival rates were significantly lower with high EZH2 expression compared with those with lower expression (48% vs. 71%; $p = 0.03$). The authors also found that high EZH2 expression was associated with thicker primary melanomas (mean Breslow thickness of 4.4 and 3.6 mm for the subgroups with high and low EZH2 expression, respectively). In contrast, in our group of non-metastatic invasive melanomas, we did not detect a significant difference in EZH2 expression between tumors less than and greater than 2.5 mm. This may be due in part to the low sample size in our group and to the inherently different population of primary invasive melanoma we studied (purely non-metastatic) compared with those studied by Bachmann et al.[11] (a sizable proportion of which metastasized).

In summary, we have identified an association of increased EZH2 protein expression with the progression of cutaneous melanoma. There was a trend toward higher EZH2 staining intensity from ordinary compound nevi and dysplastic nevi to melanoma *in situ*, although not statistically significant in this study. Invasive melanoma expressed EZH2 at significantly higher levels with respect to benign nevi and melanoma *in situ* but not as strongly as in melanoma metastatic to lymph nodes. Notably, metastatic melanomas expressed significantly higher levels of EZH2 than all other melanocytic lesions. Unfortunately, EZH2 expression in Spitz nevi overlap with melanoma, which limits the utility of EZH2 staining in distinguishing between Spitz nevi and melanoma. Nevertheless, increased expression of EZH2 in metastatic melanoma is an important finding. It may also have clinical implications in the management of patients with melanoma because the polycomb group proteins, specifically EZH2, were recently suggested as candidates for targeted treatment.[12]

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