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Original contribution

Expression of γ-H2AX in melanocytic lesions

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Summary γ-H2AX is a marker of activated DNA damage and is overexpressed in many malignancies and their precursor lesions. Previous studies have demonstrated the expression of γ-H2AX in melanoma and dysplastic nevus, but its diagnostic and prognostic utility in a full range of melanocytic lesions has not been fully studied. In the current study, we investigated γ-H2AX expression in a total of 162 melanocytic lesions. We found that γ-H2AX was observed at higher levels (percentage and intensity of staining) in melanoma in situ (12/13), primary cutaneous melanoma (32/33; with the exception of desmoplastic melanoma), and metastatic melanoma (58/62), which was statistically different from that in benign nevus (7/9), dysplastic nevus (6/10), and Spitz nevus (5/9) considered together ($P < .0001$). Of note, desmoplastic melanoma (20/26) demonstrated weak or negative γ-H2AX staining. The expression of γ-H2AX did not show significant correlation with many melanoma prognostic factors, including Breslow depth, mitotic rate, and sentinel lymph node status. Except for desmoplastic melanoma, no difference in γ-H2AX levels was observed among various melanoma subtypes. The overexpression of γ-H2AX in melanoma as opposed to nevus indicates its possible role in melanomagenesis. Based on the overlap in subsets of nevi and melanomas, the potential clinical utility of this antibody remains uncertain until further studies have been carried out in a larger cohort of melanocytic lesions, including borderline cases.

1. Introduction

Melanoma is a lethal skin cancer arising from the abnormal proliferation of epidermal melanocytes [1]. Its treatment is difficult due to its resistance to conventional radiation and chemotherapy [2]. Melanoma develops through well-defined stages that involve the loss of control of cell proliferation, acquisition of invasiveness, and ultimately metastasis [3]. The major contributing factor to melanomagenesis is thought to be ultraviolet (UV) radiation, which can induce DNA damage, followed by the development of pronounced genomic instability [4]. DNA damage can result in the activation of DNA repair pathways and trigger cell cycle arrest and/or apoptosis. Members of the phosphoinositide 3-like family of kinases, such as ataxia-telangiectasia mutated and Rad-3–related kinases, are believed to be involved in the activation of DNA damage signaling pathways. The phosphorylation of H2AX histone protein at serine 139 has been characterized as a very early event after induction of certain types of DNA damage, such as double-stranded breaks (DSBs) [5-9]. Other DNA damage repair proteins are then recruited to the sites of DSBs and are assembled into multifactor repair complexes.

γ-H2AX is an antibody that reacts specifically with phosphorylated histone H2AX and is used as a marker of activated DNA damage in cells [5]. Several studies have

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shown that γ-H2AX is expressed in cancers and their precursor lesions in organs including bladder, kidney, colon, breast, and lung [10,11]. Recently, Warters et al [6] reported that human melanoma cells express elevated levels of γ-H2AX foci (sites of DSBs) as compared to normal melanocytes. Gorgoulis et al [10] observed increased levels of γ-H2AX in melanomas, in dysplastic nevi, and in human skin xenografts (experimentally induced by growth factors), but not in normal skin.

The diagnostic criteria for melanoma are well established; however, it is sometimes difficult to make a confident distinction between dysplastic nevus and melanoma, as demonstrated by low concordance rates among dermatopathologists [12]. Over the years, many markers, such as Ki-67 (MIB-1), p16, and p53 have been shown to be helpful in differentiating melanoma from nevus [13-15]. However, to date, morphologic features and clinicopathologic correlation remain the most reliable tools in the diagnosis of melanoma.

Because γ-H2AX is involved in the cellular response to DNA damage after ionizing and/or UV radiation [7,16], we speculate that there may be a link between γ-H2AX expression and onset of melanoma. In this study, we sought to investigate the expression of γ-H2AX in a wide range of melanocytic lesions and to determine whether γ-H2AX is involved in melanomagenesis and/or melanoma progression. We also explored the diagnostic utility of γ-H2AX in the distinction between benign and malignant melanocytic lesions.

2. Materials and methods

2.1. Case selection

As approved by the Institutional Review Board at the University of Michigan Health System, a wide range of melanocytic lesions were identified through a search of the pathology database from the Department of Pathology at the University of Michigan. The study group included 9 benign nevi, 10 dysplastic nevi, 9 Spitz nevi, 13 melanoma in situ (MIS), 59 primary cutaneous melanomas (26 desmoplastic melanomas and 33 melanomas of other subtypes), and 62 metastatic melanomas. Among these, 46 metastatic melanomas and 19 desmoplastic melanomas were collected from tissue microarray sections. The primary melanomas included 14 cases with Breslow depth of less than 1.0 mm and 19 with depth of 1 mm or greater. All desmoplastic melanomas had a Breslow depth of 1.0 mm or greater. The hematoxylin and eosin–stained slides for all cases were reviewed to confirm the diagnoses. Important prognostic information for melanomas, such as depth of invasion, mitotic rate, tumor infiltrating lymphocytes, and sentinel lymph node (SLN) status, were also collected for most cases. The pathologic and clinical stages for the 33 primary cutaneous melanomas (other than desmoplastic melanomas) were determined using the American Joint Committee on Cancer staging system [17].

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were pretreated with citrate buffer at pH 6.0 for 10 minutes and were incubated with γ-H2AX antibody (phosphohistone at Ser139; 1:50 dilution; Cell Signaling Technology, Danvers, MA) for 60 minutes at room temperature on the Dako AutoStainer (Dako, Carpinteria, CA). Slides were then rinsed with buffer, incubated with rabbit EnVision+ labeled polymer for 30 minutes, and stained with DAB chromagen (Dako).

Nuclear staining was considered positive for γ-H2AX expression. The percentage of positive tumor cells was recorded as less than 10% (negative), 10% to 50% (1+), or greater than 50% (2+). The staining intensity was graded as weak, moderate, or strong. Two pathologists (MJW and LM) independently reviewed all cases and recorded the percentage and intensity of γ-H2AX staining.

2.3. Statistical analysis

Statistical analysis was carried out using SAS 8.2 software (SAS Institute, Inc, Cary, NC). The Fisher exact test and χ^2 test were used to assess the differences in the percentage and intensity of γ-H2AX staining among various melanocytic lesions. A P value less than .05 was considered statistically significant.

3. Results

γ-H2AX staining was performed on a total of 162 melanocytic lesions, including 9 benign nevi, 10 dysplastic nevi, 9 Spitz nevi, 13 MIS, 59 primary melanomas (20 superficial spreading type, 5 lentigo maligna type, 4 nodular type, 1 nevoid type, 1 acral lentiginous type, 2 unclassified type, and 26 desmoplastic type), and 62 metastatic melanomas. The clinical characteristics of these cases are listed in Table 1. For the convenience of discussion, cases of desmoplastic melanoma are separated from other primary

Table 1 The clinical characteristics of the patients

	n	Average age (range)	M/F (%)
Benign nevus	9	32 (14-46)	33/67
Dysplastic nevus	10	45 (22-72)	50/50
Spitz nevus	9	15 (5-38)	56/44
Melanoma in situ	13	63 (22-85)	79/21
Primary melanoma [a]	33	59 (34-81)	67/33
Metastatic melanoma	62	56 (21-90)	61/39
Desmoplastic melanoma	26	66 (23-94)	61/39

n, total numbers of cases; M, male; F, female.
[a] Except for desmoplastic melanoma.