Nonoverlapping Clinical and Mutational Patterns in Melanomas from the Female Genital Tract and Atypical Genital Nevi

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Genital melanomas (GM) are the second most common cancer of the female external genitalia and may be confused with atypical genital nevi (AGN), which exhibit atypical histological features but have benign behavior. In this study, we compared the clinical, histological, and molecular features of 19 GM and 25 AGN. We described chromosomal copy number aberrations and the mutational status of 50 oncogenes and tumor suppressor genes in both groups. Our study showed that a pigmented lesion occurring in mucosal tissue, particularly in postmenopausal women, was more likely to be a melanoma than a nevus. GM had high levels of chromosomal instability, with many copy number aberrations. Furthermore, we found a completely nonoverlapping pattern of oncogenic mutations when comparing GM and AGN. In GM, we report somatic mutations in *KIT* and *TP53*. Conversely, AGN had frequent *BRAF* V600E mutations, which were not seen in any of the GM. Our results show that GM and AGN have distinct clinical and molecular changes and that GM have a different mutational pattern compared with AGN.

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INTRODUCTION

Genital melanomas (GM) are the second most common cancer of the female external genitalia after squamous cell carcinoma (Micci et al., 2003) and account for 3% of all melanomas (Pappa et al., 2015). However, GM can be confused with a subset of benign melanocytic lesions that exhibit atypical histological features called atypical melanocytic nevi of genital type, or atypical genital nevi (AGN) (Barnhill et al., 2004). Histologic distinction of GM and AGN can be challenging (Barnhill et al., 2004; Clark et al., 1998). Differentiating these entities is important because of the difficulty involved in performing wide local excisions and the potential for surgical complications leading to sexual or urinary dysfunction.

Studies targeting *BRAF*, *NRAS,* and *KIT* have shown that vulvovaginal melanomas, in contrast to conventional cutaneous melanomas, typically harbor wild-type *BRAF*, whereas *KIT* and *NRAS* mutations are present in approximately 20% of patients (Heinzelmann-Schwarz et al., 2014; Tseng et al., 2014; van Engen-van Grunsven et al., 2014). Conversely, separate studies analyzing the same genes in AGN have shown frequent *BRAF* mutations, particularly V600E (Nguyen et al., 2010). In the last decade, next-generation sequencing has expanded our knowledge of the genes implicated in different subsets of melanomas, but few studies have used this technique to analyze GM (Furney et al., 2013; Krauthammer et al., 2012).

In this study, we sought to compare the clinical, histological, and molecular features in a series of 19 melanomas from the female genital tract and 25 AGN. We assessed chromosomal copy number aberrations in GM using fluorescence in situ hybridization (FISH) and evaluated for the presence of mutations in 50 oncogenes and tumor suppressor genes in both GM and AGN using the Ion Torrent Personal Genome Machine (PGM) (Life Technologies, Grand Island, NY) and AmpliSeq Cancer Hotspot Panel, v2 (Life Technologies). We saw no differences in the molecular patterns of genital nevi from hair-bearing versus non–hair-bearing skin. Similarly, GM, whether from mucosal or hair-bearing epithelium, showed a mutational profile that was distinct from AGN and most melanomas of sun-exposed skin. Therefore, our results suggest that GM and AGN arise through distinct molecular changes.

RESULTS

Clinical findings

We reviewed clinical and histological information for 19 GM and 25 AGN patients (Table 1). The median age of patients with GM was 63 years (range = 40–94 years), compared with 29 years (range = 8–42 years) for patients with AGN ($P < 0.0001$). Most GM occurred in postmenopausal women (16/19), whereas no cases of AGN occurred in

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Abbreviations: AGN, atypical genital nevi; FISH, fluorescence in situ hybridization; GM, genital melanoma; PGM, personal genome machine; SIFT, Sorting Intolerant from Tolerant algorithm

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Table 1. Clinical and histological findings in genital melanomas and atypical genital nevi

Finding	GM	AGN	*P*-Value[1]
Median age, years (range)	63 (40–94)	29 (8–42)	$P < 0.0001$
Postmenopausal, n (%)	16/19 (81.2)	0/23 (0)	$P < 0.0001$
Vulvar location, n inner:outer	11:8	5:20	$P = 0.0133$
Tissue type, n			$P < 0.0001$
Glabrous + mucosa	14	3	
Hair-bearing skin	5	22	
Median size, cm (range)	2 (0.4–5)	0.7 (0.3–1.5)	$P = 0.0003$
Multiple:single lesion(s), n (%)	4:15 (26.7)	0:25 (0)	$P = 0.0286$
Raised:flat, n	11:8	10:14	$P = 0.364$
Ulceration, n	12/18	1/25	$P < 0.0001$
Prominent intraepithelial component, n	14/18	25/25	$P = 0.025$
Pagetoid spread, n	10/18	1/25	$P < 0.0001$
Lentiginous growth, n	14/18	17/25	$P = 0.732$
Consumption of epidermis, n	13/17	7/25	$P = 0.004$
Confluent nesting, n	9/18	21/25	$P = 0.023$
Mitoses/mm^2, median (range)	5 (0–30)	0 (0–1)	$P < 0.0001$
Nuclear atypia, n high:low	16:2	0:25	$P < 0.0001$

Abbreviations: AGN, atypical genital nevi; GM, genital melanoma.
[1]*P*-values less than 0.003 were considered statistically significant after correcting for multiple comparisons.

postmenopausal women ($P < 0.0001$). GM were also more commonly mucosal ($P < 0.0001$) and significantly larger in size ($P = 0.0003$), whereas AGN were generally smaller and more frequently occurred on hair-bearing skin.

Follow-up information was available for all patients with GM (median = 21 months, range = 5–137 months). Nine patients had lymph node involvement, primarily detected during histological assessment of lymph nodes excised during surgical resection of the melanoma. Distant metastases were found in 13 patients, with most (n = 9) involving multiple anatomic regions, including the liver (n = 9), lungs (n = 5), bone (n = 2), brain (n = 2), bladder (n = 2), and distant skin (n = 2). All patients with metastatic melanoma died of disease (median = 26 months, range = 6–75 months), and three additional GM patients died from events unrelated to their melanoma. The remaining three GM patients were alive at the last follow-up with no evidence of active disease (median = 119 months, range = 11–141). Follow-up information was available for 21 of 25 (84%) patients with AGN; all patients were alive at the last follow-up visit and had no evidence of recurrence after complete excision (median = 34 months, range = 2–62 months).

Histological and FISH findings

The median Breslow depth among patients with primary GM was 3.5 mm (range = 0.45–38 mm). Ulceration was noted in 12 of 18 patients with GM. Most patients had a prominent intraepithelial component (14/18), and pagetoid spread was seen in 10 patients (Figure 1a). Fourteen patients showed prominent lentiginous growth. Confluent nesting was noted in half of the patients with GM. Consumption of the epidermis/epithelium was noted in 13 patients with melanomas. Most patients with GM had severe nuclear atypia

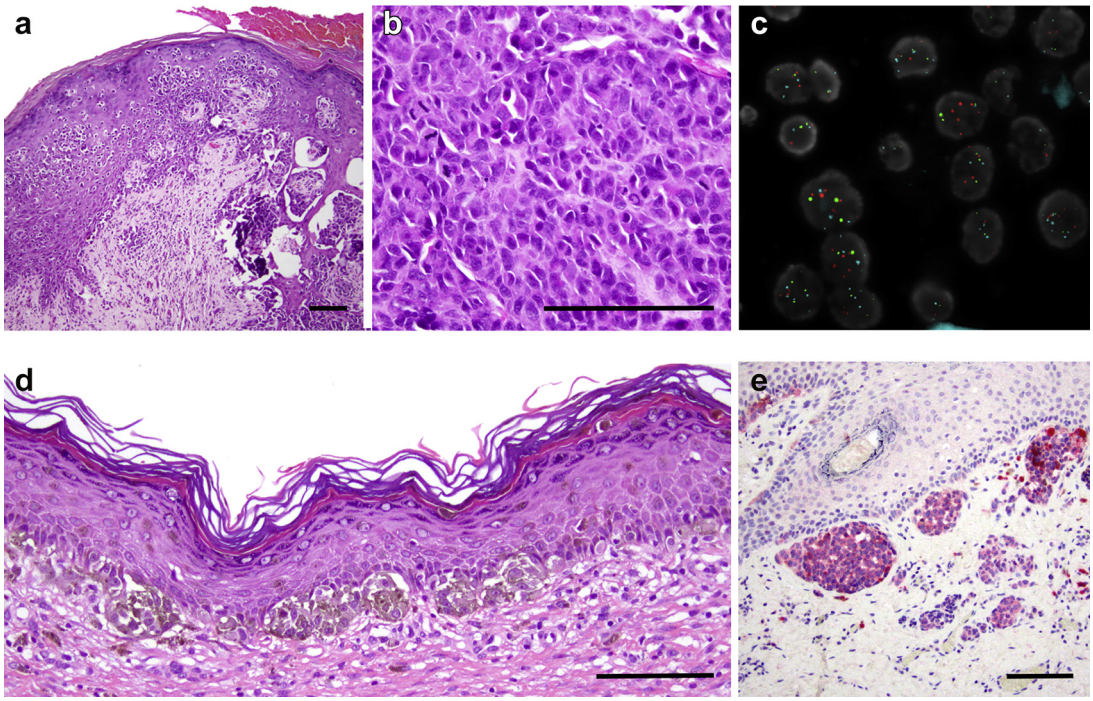

Figure 1. Histological and fluorescence in situ hybridization findings in a genital melanoma and in an atypical genital nevus. (**a**) Low-power magnification shows extensive epithelial ulceration, extensive pagetoid spread, and prominent lentiginous growth in a genital melanoma. (**b**) At high-power magnification, the cells have severe nuclear atypia with pleomorphism, coarse chromatin, and high mitotic activity. (**c**) Fluorescence in situ hybridization showed numerous copy number gains involving chromosome bands 6p25 (red), 8q24 (aqua), and 11q13 (green). (**d**) Atypical genital nevus showing extensive junctional involvement with effacement of the rete ridges, back-to-back nesting, and focal pagetoid spread. (**e**) The *BRAF* VE1 immunostain showed that the cells in the atypical genital nevus harbored the *BRAF* V600E mutation. Scale bar = 100 μm.