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***BRAF* V600E mutation and the tumour suppressor IGFBP7 in atypical genital naevi**

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Summary

Background—Atypical genital naevi (AGN) are naevi of special sites with atypical histological features that overlap with those of malignant melanoma. Activating *BRAF* mutations, identified in the majority of banal melanocytic naevi and cutaneous melanomas, are reportedly uncommon in naevomelanocytic proliferations in nonsun-exposed sites. We have recently shown that constitutive activation of the *BRAF*-*MEK*-*ERK* signalling pathway in oncogenic *BRAF*-positive naevi increases expression and secretion of IGFBP7, which induces senescence and apoptosis.

Objectives—To ascertain the frequency of *BRAF*V600E mutations in AGN compared with banal naevi without atypia. An additional aim was to assess the expression of IGFBP7 in oncogenic *BRAF*-positive AGN.

Methods—Genomic DNA was isolated per protocol from seven genital naevi without atypia and 13 AGN for *BRAF* genotyping. Immunohistochemical staining for IGFBP7 was performed on all cases.

Results—The *BRAF*V600E mutation was identified in 43% of genital naevi without atypia and 23% of AGN ($P = 0.61$). In both groups, IGFBP7 expression was maintained in 67% of *BRAF* V600E-positive cases.

Conclusions—The prevalence of *BRAF*V600E in AGN suggests that ultraviolet exposure is not essential for generating the mutation. The *BRAF*V600E mutational status appears to be of limited diagnostic utility in distinguishing genital naevi that exhibit atypia from those that do not. Similar to oncogenic *BRAF*-positive common naevi without atypia, enhanced expression of the tumour suppressor IGFBP7 in oncogenic *BRAF*-positive AGN supports that they are biologically inert.

Keywords

atypical genital naevi; BRAF V600E; IGFBP7

First described by Clark *et al.*, atypical genital naevi (AGN) are a relatively uncommon subset of clinically benign naevi of the genitalia or perineum that exhibit atypical histological features.^{1,2} Similar lesions have subsequently been described along the

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Conflicts of interest

None declared.

anatomical milk line including the groin area.³ Unifying histological features of AGN include variably sized nests with retraction artefact, consumption of the epidermis, cellular dyshesion, dermal fibrosis and cytological atypia ranging from mild to moderate severity.¹ As these features are concerning for melanoma, overdiagnosis of AGN is not uncommon and may lead to unnecessary surgical intervention and sentinel lymph node biopsy.¹ This is of particular concern given that these naevi typically occur in women of childbearing age.^{1,2}

No study to date has sought to elucidate the genomic profile of AGN as compared with benign genital naevi without atypia. We recently found that in oncogenic *BRAF*-positive naevi, constitutive activation of the *BRAF-MEK-ERK* pathway increases expression and secretion of IGFBP7,⁴ a protein that suppresses the growth of a range of cancer cell types.^{5,6} We also noted that IGFBP7 expression is maintained or enhanced in *BRAF* wild-type (WT) melanomas but is not detectable in oncogenic *BRAF*-positive melanomas. Based on these findings, we concluded that IGFBP7 inhibits *BRAF-MEK-ERK* signalling and activates senescence via an autocrine/paracrine pathway.

Therefore, the primary goal of this study was to assess the frequency of *BRAF*V600E mutations in AGN and to compare with that in banal naevi of genital skin. Given our recent experience with IGFBP7, an additional goal was to assess IGFBP7 expression in oncogenic *BRAF*-positive AGN.

Materials and methods

This study was approved by Boston University School of Medicine institutional review board (IRB docket H27806). Archival materials between 2006 and 2008 with a histological diagnosis of 'naevus' ($n = 7$) and 'naevus with atypia' ($n = 13$) located in the genital area [mons, pubic and suprapubic ($n = 11$), labia ($n = 4$), perineum ($n = 1$), groin ($n = 3$) and scrotum ($n = 1$)] were retrieved from the pathology files of the Skin Pathology Laboratory (Boston University School of Medicine, Boston, MA, U.S.A.). All patient data were de-identified. All histological sections were re-reviewed and diagnoses confirmed by the dermatopathologist (M.M.). Five of seven (71%) cases of genital naevi without atypia and 12 of 13 (92%) cases of AGN were female. Median age was 33.5 years (range 11.5–76.6) and 33.4 years (range 14.7–50.1) for each group, respectively.

Microscopic examination of the 13 AGN revealed compound naevi exhibiting varying degrees of one or more of the following: variably sized nests with retraction artefact and/or consumption of the epidermis, cellular dyshesion, and dermal fibrosis. Severity of cytological atypia ranged from mild ($n = 2$) to moderate ($n = 8$) and severe ($n = 3$). All seven cases of genital naevi without atypia were compound naevi with features of a congenital naevus.

Mutational analysis

DNA was extracted by proteinase K digestion of microdissected samples per protocol. Briefly, 5- to 7- μ m thick sections of formalin-fixed paraffin-embedded archival tissue were deparaffinized and rehydrated prior to microdissection. Direct DNA sequencing was performed on exon 15 of the *BRAF* gene (forward strand) spanning codon 600 using an ABI BigDye TerV3.1 cycle sequencing terminator ready reaction kit (Applied Biosystems, Inc., Foster City, CA, U.S.A.). The sequence of the forward primer was 5'-TCATAATGCTTGCTCTGATAGGA-3'. Sequencing reactions were performed on an ABI 9700 thermocycler utilizing the ABI recommended protocol and analysed with the Genetic Analyzer 3100-avant (ABI). The sequencing results were analysed with ABI DNA Sequencing Analysis Software version 3.7. A positive and/or negative control was included.

IGFBP7 immunohistochemistry

Immunohistochemical studies were performed on 5- μ m thick sections using standard peroxidase immunohistochemistry techniques, heat-induced epitope retrieval buffer and a primary antibody against IGFBP7 (C-16; 1:20; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, U.S.A.). Appropriate positive and negative controls were used. Positive expression of IGFBP7 in the cytoplasm was scored as 1+ (< 10% of tumour cells), 2+ (10–50%) and 3+ (> 50%). Cases with an immunohistochemical score of 1+ were considered negative while those with a score of 2+ or 3+ were considered positive. Significant nuclear staining was not observed. All stained slides were initially reviewed and scored by the first author (L.P.N.) and were re-reviewed by the dermatopathologist (M.M.) in a blinded fashion with respect to genotype. Disagreements were reviewed together to achieve a consensus score.

Statistical analysis

Fisher's exact test was used to compare categorical variables. An alpha of 0.05 was used as the level of significance.

Results

Data from genotyping and expression of tumour suppressor IGFBP7 are summarized in Table 1, and representative cases are illustrated in Figure 1.

BRAF mutational status

The *BRAF*V600E allele was detected in three of 13 (23%) AGN and three of seven (43%) genital naevi without atypia ($P = 0.61$). All three AGN expressing the *BRAF*V600E mutation demonstrated a moderate degree of cytological atypia.

IGFBP7

Of the cases of AGN, cytoplasmic staining (3+) was observed in two of three cases (67%) that were *BRAF*V600E-positive and in eight of 10 cases (80%) that were *BRAF*WT. Of the cases of genital naevi without atypia, cytoplasmic staining (3+) was observed in two of three cases (67%) that were *BRAF*V600E-positive and in four of four cases (100%) that were *BRAF*WT. No significant association was found between *BRAF* mutational status and IGFBP7 expression in AGN or genital naevi without atypia ($P = 0.58$ and $P = 0.43$, respectively).

Discussion

Oncogenic *BRAF* mutations are known to predispose to melanoma with a reported attributable risk of 1.6% and have been found in both melanocytic naevi and melanoma.^{7,8} However, the mutation is not present in all types of melanoma, and differences in incidence have been attributed to aetiological factors including sun exposure.⁹ In keeping with this, *BRAF* mutations have been documented in 62% of melanomas arising in sun-exposed sites compared with 0% of vulval melanomas, suggesting that distinct molecular pathways may be involved depending on the presence or absence of sun exposure.⁹ However, the *BRAF* V600E mutation was identified in 23% and 43% of AGN and genital naevi without atypia, respectively, in the current study. Ichii-Nakato *et al.* also found the presence of the *BRAF* V600E mutation in up to 81% of acquired naevi from glabrous skin.¹⁰ The higher proportion may be attributable to different methodologies used in mutation analysis (Mutector assay vs. direct sequencing in the current study) and the cohort studied (acquired naevi without atypia vs. congenital naevi and naevi with atypia in the current study). None the less, both studies support the concept that ultraviolet (UV) radiation is not essential for acquisition of the *BRAF* mutation. While the clinical relevance of *BRAF* mutations in

naevomelanocytic proliferations in non-sunexposed skin remains unclear, somatic mutations of *BRAF* have previously been identified as the most common early genetic event causally associated with development of papillary thyroid cancer in patients without a history of radiation exposure.¹¹ This implicates other environmental or intrinsic biological factors as potential causative agents for *BRAF* mutations.

AGN pose a significant diagnostic conundrum. Exemplifying this, one of the cases in the current study was that of a 'moderate to severely atypical compound neoplasm' in a 14-year-old patient. The report noted that 'although the lesion exhibited maturation, given the degree of cytological atypia, florid junctional melanocytic proliferation and presence of dyscohesive nests, malignant melanoma, level III could not be excluded' by five experienced dermatopathologists. No study to date has sought to elucidate the genomic profile of AGN as compared with benign genital naevi without atypia. We found no significant difference in the frequency of oncogenic *BRAF* in AGN compared with naevi without atypia from the same anatomical location. Thus, the utility of oncogenic *BRAF* as a diagnostic adjunct appears to be limited. However, a larger study is needed before a definitive conclusion can be reached.

We have previously found that normal melanocytes express detectable, albeit low, levels of IGFBP7.⁴ *In vitro* evidence indicates that IGFBP7 blocks *BRAF-MEK-ERK* signalling to activate the apoptotic pathway, suggesting that it is an inducer of senescence and apoptosis. Consistent with this, we also found IGFBP7 expression to be upregulated in *BRAF*^{WT} melanomas and oncogenic *BRAF*^{V600E}-positive naevi, but absent in *BRAF*^{V600E}-positive melanoma. In the current study, the enhanced expression of IGFBP7 found in 67% of oncogenic *BRAF*-positive AGN suggests that these lesions are biologically inert. While the significance of our findings is limited by the small number of cases studied, oncogenic *BRAF*-positive AGN cases with loss of IGFBP7 expression may warrant complete excision and close clinical follow-up.

In conclusion, the prevalence of *BRAF*^{V600E} in AGN suggests that UV exposure is not essential for generating the *BRAF*^{V600E} mutation. Furthermore, AGN appear not to differ from genital naevi without atypia with respect to *BRAF* mutational status. Enhanced expression of the tumour suppressor IGFBP7 in oncogenic *BRAF*-positive AGN suggests that these lesions behave similarly to oncogenic *BRAF*-positive naevi. Larger studies and long-term follow-up of AGN is required to confirm their putative banal biological behaviour.

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Fig 1.

Composite of genomic and immunohistochemical staining with representative cases with/without *BRAF*V600E mutation and positive/negative IGFBP7 expression. (a) Atypical genital naevus with moderate cytological atypia, with (b) *BRAF*V600E mutation and (c) positive IGFBP7 expression. (d) Atypical genital naevus with moderate cytological atypia, with (e) *BRAF*V600E mutation and (f) negative IGFBP7 expression. (g) Atypical genital compound melanocytic neoplasm with moderate to severe atypia, with (h) *BRAF*wild-type (WT) and (i) positive IGFBP7 expression. (j) Compound melanocytic naevus with features suggestive of congenital onset, with (k) *BRAF*V600E mutation and (l) positive IGFBP7 expression. (a, d, g, j) Haematoxylin and eosin; original magnification $\times 20$.

Table 1

Summary of *BRAF* mutational status and IGFBP7 positivity in genital naevi

<i>Atypical genital naevi</i>		
No. of cases/total (%)	<i>BRAF</i> WT (<i>n</i> = 10)	<i>BRAF</i> V600E (<i>n</i> = 3)
IGFBP7 positive	8/10 (80)	2/3 (67)
IGFBP7 negative	2/10 (20)	1/3 (33)
<i>Genital naevi without atypia</i>		
No. of cases/total (%)	<i>BRAF</i> WT (<i>n</i> = 4)	<i>BRAF</i> V600E (<i>n</i> = 3)
IGFBP7 positive	4/4 (100)	2/3 (67)
IGFBP7 negative	0/4 (0)	1/3 (33)

WT, wild-type.