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Original contribution

Molecular testing of borderline cutaneous melanocytic lesions: SNP array is more sensitive and specific than FISH☆

Michael D. Carter MD, PhD[a,*,1], Alison B. Durham MD[b], Jayson R. Miedema MD[a], Paul W. Harms MD, PhD[a,b], May P. Chan MD[a,b], Rajiv M. Patel MD[a,b], Lori Lowe MD[a,b], Douglas R. Fullen MD[a,b], Alexandra C. Hristov MD[a,b], Min Wang PhD[a], Aleodor A. Andea MD, MBA[a,b]

[a]*Department of Pathology, Michigan Medicine, University of Michigan, Ann Arbor, MI, USA 48109-5602*
[b]*Department of Dermatology, Michigan Medicine, University of Michigan, Ann Arbor, MI, USA 48109-5602*

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Summary Melanocytic lesions with borderline features are diagnostically challenging. Single-nucleotide polymorphism (SNP) arrays, which detect genomic copy number alterations (CNAs), can be helpful in distinguishing between nevi and melanoma. Fluorescence in situ hybridization (FISH) has been used as a more rapid, less expensive alternative to SNP array, using a panel of probes that are often gained or lost in melanoma. We used SNP array data from 63 borderline cutaneous melanocytic lesions and 44 definitive melanomas to predict the performance of FISH testing. Lesions were considered positive by "virtual FISH" if 1 or more of the 5 FISH-probed loci demonstrated appropriate CNAs by SNP array. Cases were classified as positive by SNP array if ≥3 CNAs were present, based on internal validation studies, or if FISH criteria were met. Conventional FISH was performed in 33 cases (17 borderline lesions, 16 melanomas). Of the 63 borderline cases, 44 (70%) were positive by SNP array and 30 (48%) were positive by virtual FISH. A higher proportion of melanomas were positive by SNP array (41/44, 93% sensitivity) and virtual FISH (36/44, 82% sensitivity). Virtual FISH had 61% sensitivity in the borderline group using SNP array as the gold standard, whereas specificity was 84%. There was good correlation between conventional and virtual FISH, with agreement in 30 of 33 (91%) cases. Although FISH is highly effective in distinguishing between nevi and melanoma in cases where the histological diagnosis is straightforward, it is not nearly as sensitive or specific as SNP array when applied to borderline lesions.

☆ Disclosures: none

* Corresponding author at: Michigan Medicine Department of Pathology, 3261 Medical Science I, 1301 Catherine St, Ann Arbor, MI, USA 48109-5602.
E-mail addresses: michacar@med.umich.edu, michaeld.carter@nshealth.ca (M. D. Carter), ambates@med.umich.edu (A. B. Durham), miedemaj@med.umich.edu (J. R. Miedema), paulharm@med.umich.edu (P. W. Harms), mpchan@med.umich.edu (M. P. Chan), rajivpat@med.umich.edu (R. M. Patel), lorilowe@med.umich.edu (L. Lowe), dfullen@med.umich.edu (D. R. Fullen), ahristov@med.umich.edu (A. C. Hristov), minw@med.umich.edu (M. Wang), andeaa@med.umich.edu (A. A. Andea).
[1] Current address: Division of Anatomical Pathology, Queen Elizabeth II Health Sciences Centre and Dalhousie University, Mackenzie Bldg, 5788 University Ave, 7th floor, Halifax, Nova Scotia, Canada B3H 1V8.

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1. Introduction

Melanoma is an aggressive cutaneous malignancy with high mortality and increasing incidence that derives from pigment-producing melanocytes, the behavior of which is under local and hormonal regulation [1]. Although histological diagnosis of melanoma is straightforward in most cases, in a small but significant subset of borderline melanocytic proliferations, definitive classification remains a major challenge. These are lesions with a degree of architectural and/or cytological atypia that exceeds what is normally expected in nevi but is insufficient to reach a diagnosis of melanoma. Their behavior is difficult to predict, which can lead to suboptimal management in the form of under- or overtreatment [2].

Even expert dermatopathologists struggle with categorizing borderline melanocytic lesions as either benign or malignant, with studies consistently finding poor inter- and intraobserver reproducibility and low sensitivity and specificity [2-4]. For example, one study found that, of 57 borderline tumors with clinical follow-up, the expert panel had only 73% sensitivity and 47% specificity in diagnosing melanoma [2]. Challenges in diagnosing melanocytic tumors include distinguishing dysplastic nevi from superficial spreading melanoma, Spitz nevi from spitzoid melanoma, ordinary nevi from nevoid melanoma, and atypical blue nevi from blue nevus–like melanoma [5].

The costs associated with a misdiagnosis of a nevus as melanoma, including additional surgery for wider margins, lymph node biopsy in some cases, and the psychological burden borne by the patient, are substantial. The opposite scenario, that is, misdiagnosis of a melanoma as a nevus, can result in disease recurrence and progression, in some cases resulting in metastatic disease and death that might have been prevented by timely diagnosis [6]. Both scenarios (over- and undertreatment) highlight the need for improved tools in distinguishing benign from malignant melanocytic lesions.

A number of ancillary techniques have been developed to help predict whether a melanocytic lesion with borderline features is likely to behave in an indolent or aggressive manner. Molecular assays that evaluate chromosomal copy number are useful for this distinction, as the majority of melanomas, but not nevi, display genomic instability resulting in multiple copy number aberrations (CNAs) [7,8]. Comparative genomic hybridization (CGH) identifies CNAs across the tumor genome, with deleterious DNA losses (eg, homozygous loss of *CDKN2A*) or gains (eg, high-level amplification of *MYC*), or a pattern of several CNAs being consistent with malignancy [8,9]. By contrast, most benign melanocytic lesions either demonstrate no CNAs or have isolated abnormalities such as gain of 11p that by themselves are rare in melanoma [8]. In addition, gains and/or losses of entire chromosomes without segmental abnormalities can be seen in benign melanocytic proliferations, including proliferative nodules, and are seldom encountered in melanomas [10]. More recent versions of CGH incorporate probes with single-nucleotide polymorphisms (SNPs). So-called "SNP arrays" provide allelic information and thereby identify not only genomic gains and losses in the tumor but also regions with copy-neutral loss of heterozygosity [11,12].

Fluorescence in situ hybridization (FISH) has been used as a more rapid, less expensive alternative to SNP array, using a panel of probes to genes that tend to be gained or lost in melanoma [13]. Although the sensitivity and specificity of FISH testing are both reported to be in excess of 90%, validation studies are based on well-characterized nevi and melanomas rather than borderline melanocytic tumors.

In this study, our aim was to compare FISH to SNP array in predicting the behavior of a cohort of borderline melanocytic tumors. Although conventional FISH was performed in a subset of cases, the main focus was on "virtual FISH," which predicts FISH results based on SNP array data.

A previous study has compared the performance of FISH to array CGH on a cohort of melanocytic lesions, similar to the design of our study, but it included only unambiguous nevi and melanomas [14]. To the best of our knowledge, ours is the first study to compare the performance of the 2 technologies, CGH/SNP array and FISH (including both conventional and virtual FISH), on a large and diverse set of borderline melanocytic lesions.

2. Materials and methods

2.1. Specimen cohort

Institutional review board approval was obtained for the study. Formalin-fixed, paraffin-embedded (FFPE) skin biopsies and excisions of borderline primary cutaneous melanocytic lesions (n = 63) and definitive melanomas (n = 44) were identified from the University of Michigan surgical pathology archives. Cases were mostly outside consults received between 2011 and 2018. The borderline cohort consisted of lesions which were histopathologically diagnosed as atypical Spitz tumor (n = 36), melanocytic tumor of uncertain malignant potential (n = 23), atypical deep penetrating nevus of uncertain malignant potential (n = 2), atypical cellular blue nevus of uncertain malignant potential (n = 1), and pigmented epithelioid melanocytoma (n = 1). The definitive melanoma cohort consisted of lesions histopathologically diagnosed as malignant melanoma not otherwise specified (n = 14), nodular melanoma (n = 11), metastatic melanoma (n = 9), superficial spreading melanoma (n = 4), desmoplastic melanoma (n = 3), blue nevus–like melanoma/melanoma ex blue nevus (n = 2), and acral lentiginous melanoma (n = 1). Noncutaneous primary melanomas were not included.

2.2. Fluorescence in situ hybridization

Conventional FISH was performed in 33 cases as described [15]. Briefly, a set of 7 Vysis Melanoma-FISH probes (Abbott Molecular, Des Plaines, IL) for *RREB1* (6p25), CEP6, *MYB* (6q23), *MYC* (8q24), *CDKN2A* (9p21), CEP9, and *CCND1*

Table 1 Clinical characteristics and SNP array and virtual FISH results for borderline melanocytic lesions and definitive melanomas

	Borderline (n = 63)	Melanoma (n = 44)	*P*
Median age, y	21.4	63.6	1×10^{-14}
Female, %	63.5	38.6	.018
SNP array positive (%)	44 (70)	41 (93)	.003
Virtual FISH positive (%)	30 (48)	36 (82)	.0005

(11q13) was used according to the manufacturer's instructions. Following established criteria from internal validation and published data [13], cases were interpreted as positive for melanoma if any FISH abnormality was present at the following levels in scored cells: gain of 6p25 (>29% of cells), 8q24 (>29%), or 11q13 (>38%) or homozygous loss of 9p21 (>29%). Additionally, a 6q23:CEP6 ratio of less than 1 in >40% of cells was considered positive. Cases in which immunohistochemical studies were performed showing retention of p16 expression were not always probed for *CDKN2A*,

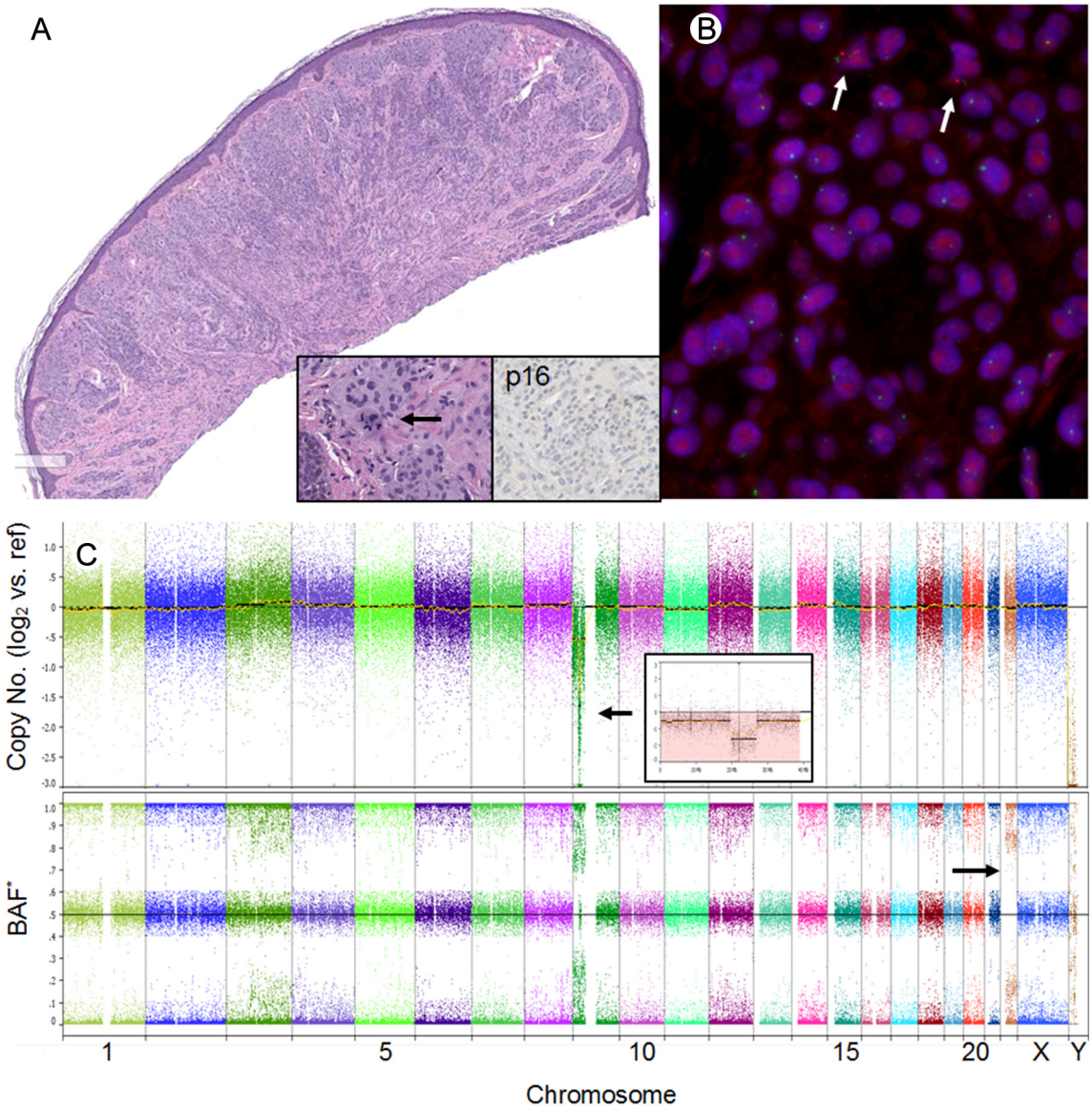

Fig. 1 Nevoid melanoma from the shoulder of a 40-year-old woman. A, H&E (original magnification ×20) slide showing a small, slightly asymmetric, and well-circumscribed melanocytic lesion composed of small to intermediate-sized melanocytes. Increased mitotic activity was noted, including occasional bizarre/atypical mitoses (inset, arrow, ×400). There were also diffuse loss of p16 immunoexpression (second inset, ×400) and increased Ki67 labeling index (data not shown). B, FISH shows homozygous loss of 9p21 (red probe, arrows indicate rare retained signals) and heterozygous loss of CEP9 (green, 1 signal per cell) in most cells (×1000). C, SNP array shows heterozygous loss of 9p and focal homozygous loss of *CDKN2A* at 9p21 in the upper panel, which depicts copy number data (short arrow and inset). There is also copy neutral loss of heterozygosity of chromosome 22, evident in the signal splitting seen in the lower panel (long arrow), which displays zygosity data. Abbreviation: BAF, B allele frequency.

as homozygous loss of this locus in such cases would be very unlikely [16].

2.3. SNP array and "virtual FISH"

Thick sections (10 umol/L, n = 2–10) were cut from a representative FFPE tissue block, and tumor was macrodissected using a hematoxylin and eosin (H&E)–stained slide as a guide. DNA was extracted and purified from the samples using the QIAmp DNA FFPE Tissue Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer's protocols. Extracted DNA was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Genomic CNA studies were performed using the OncoScan FFPE Express

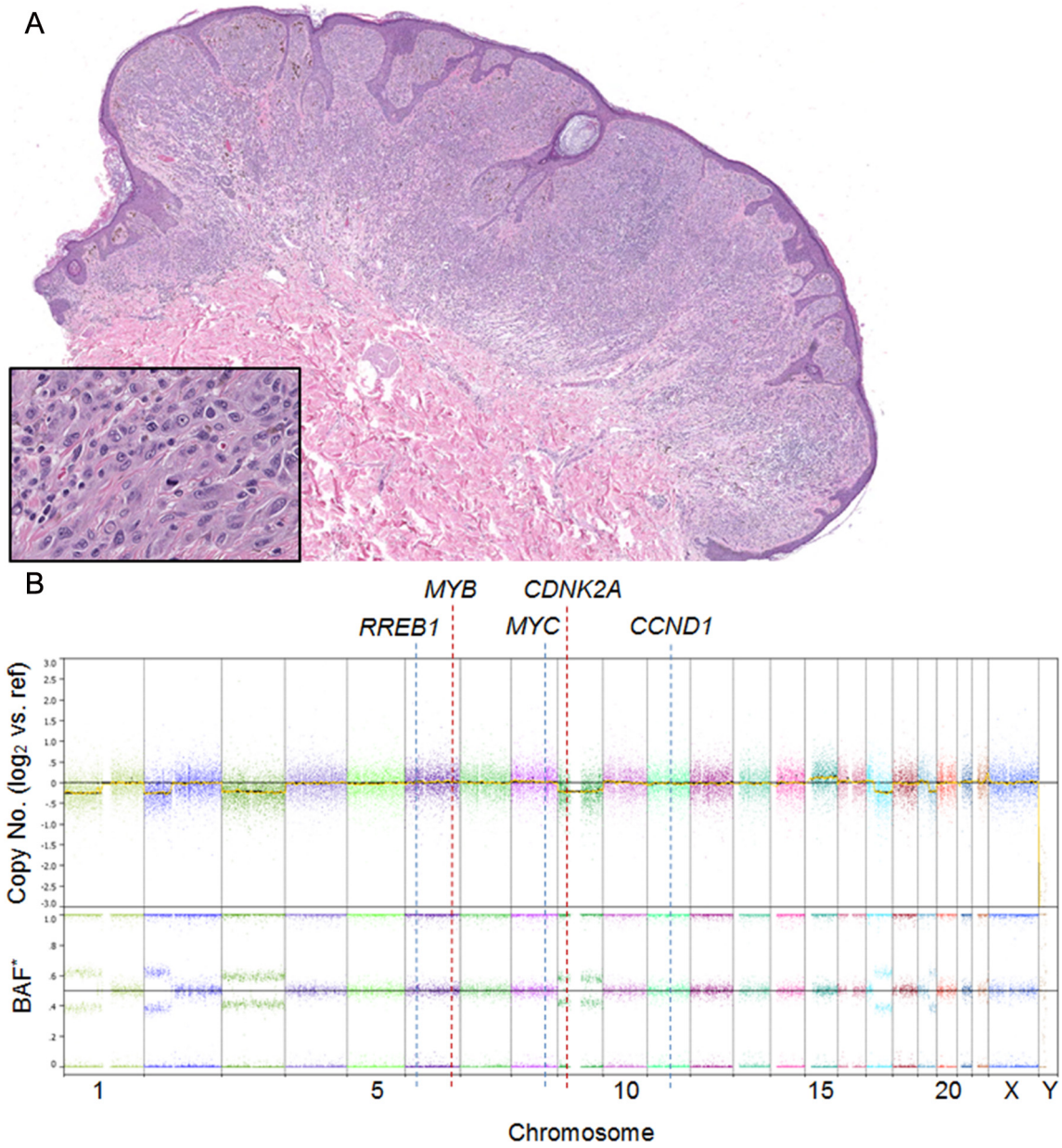

Fig. 2 Borderline compound spitzoid melanocytic proliferation from the back of a 37-year-old woman. A, H&E sections show nests of atypical epithelioid to spindled melanocytes expanding dermal papillae; occasional mitotic figures are identified (inset). Ki67 proliferation index was mildly elevated (5%-10%), and p16 and BAP-1 immunoexpression were both retained (data not shown). B, Concerning SNP array (upper panel: copy number data, lower panel: allelic ratio plot) showing multiple chromosomal abnormalities, including partial loss of chromosomes 1p, 2p, 17q, and 19q; loss of whole chromosomes 3 and 9; partial gain of chromosome 22q; and gain of whole chromosome 15 (all gains and losses heterozygous). None of the FISH probe locations (dotted lines) are aberrant (loss of *CDKN2A* is heterozygous, consistent with retention of p16 immunostaining); hence, virtual FISH is negative for this case despite positive SNP array.

3.0 gene chip probe SNP microarray (Affymetrix, Santa Clara, CA), as described [11,12,17]. Briefly, DNA from each sample (80 ng) was probed with more than 220,000 SNP-containing molecular inversion probes targeting interrogation sites of 40 base pairs. Probe fluorescence was compared with a reference human genome, yielding information on both copy number changes (gains and losses) and allelic frequency/zygosity.

Copy number alteration data from each sample were analyzed using Nexus Copy Number software version 9.0 (BioDiscovery, El Segundo, CA). Diploid correction was performed, when necessary, according to the manufacturer's instructions. Clinical quality control threshold of median absolute pairwise difference (MAPD) <0.3 was applied, and cases not meeting this cutoff were not included in the study, except

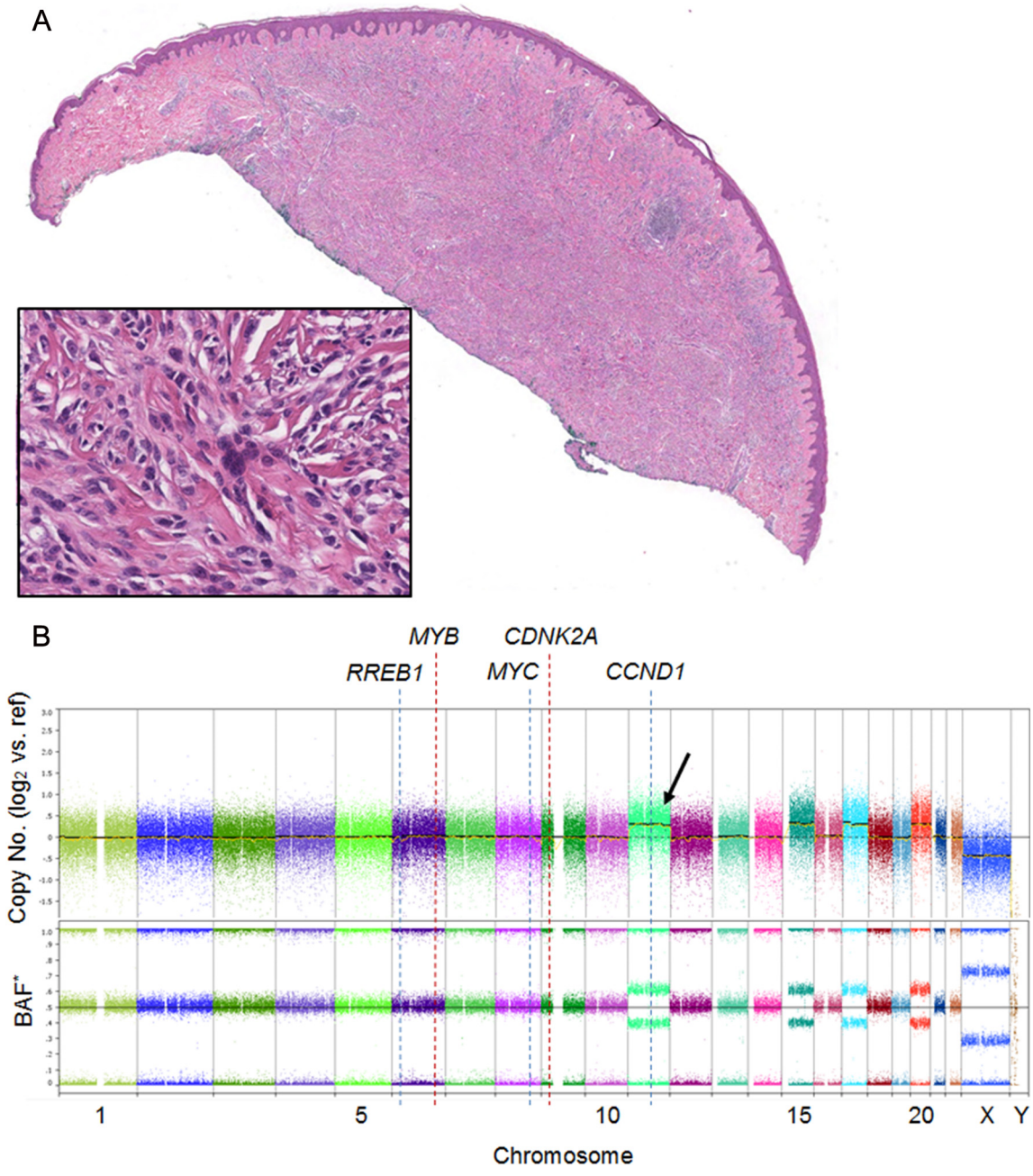

Fig. 3 Atypical compound desmoplastic spitzoid melanocytic proliferation arising on the left elbow of a 31-year-old woman. A, H&E sections show a symmetric and circumscribed dome-shaped nodule composed of small nests of melanocytes with pleomorphic and hyperchromatic nuclei, occasionally prominent nucleoli, and rare mitoses. B, SNP array shows gains of whole autosomes (chromosomes 11, 15, 17, and 20) and heterozygous loss of the entire X chromosome, consistent with a benign lesion. Gain of chromosome 11 (arrow) triggers a positive result by virtual FISH, which was confirmed by gain of the 11q13 (*CCND1*) probe on conventional FISH (data not shown).

Table 2 Correlation between SNP array and virtual FISH results, with the former acting as gold standard

Group	Virtual FISH		FISH sensitivity	FISH specificity	SNP array–FISH agreement
	Positive	Negative			
Definitive melanomas (n = 44)					
SNP array					
Positive	36	5	82%	100%	89%
Negative	0	3			
Borderline (n = 63)					
SNP array					
Positive	27	17	61%	84%	68%
Negative	3	16			

in instances where definitive determination could be made with regard to CNA and FISH criteria. Lesions were considered positive for melanoma by virtual FISH if 1 or more of the 5 FISH-probed loci demonstrated appropriate CNAs by SNP array: gain of 6p25, 8q24, or 11q13, or loss of 6q23 (heterozygous or homozygous) or 9p21 (homozygous loss only). Cases were classified as positive by SNP array if ≥3 CNAs were present, based on internal data showing occasional nevi with up to 2 CNAs, or if virtual FISH criteria were met. Only those gains or losses involving a region >3 Mb and loss of heterozygosity >10 Mb were included in the CNA count. If ≥3 CNAs were present but all involved gain or loss of an entire chromosome, regardless of whether FISH loci were aberrant, this was deemed to be consistent with a benign proliferative nodule [10] and hence considered a negative result. For virtual FISH, no lower size limit was imposed on gains, provided the entire gene was gained, nor on losses involving *MYB* or *CDKN2A*. These determinations were made on a case-by-case basis by the reviewer (M. D. C.), with consensus obtained from a second reviewer (A. A. A.) in difficult cases.

2.4. Statistics

χ^2 and 2-sided *t* tests were used to identify differences between the borderline and definitive melanoma groups using a threshold of $P < .05$.

3. Results

Demographic and clinical data for patients with borderline melanocytic lesions and definitive melanomas are provided in Table 1. The borderline cohort differed substantially from the melanoma group, having a much younger median age (21.4 versus 63.6 years, $P = 1 \times 10^{-14}$) and consisting of a higher proportion of female patients (63.5% versus 38.6%, $P = .018$). The frequencies of SNP array positivity (93% versus 70%, $P = .003$) and FISH positivity (82% versus. 48%, $P = .0005$) were both higher in the definitive melanoma group than in the borderline group.

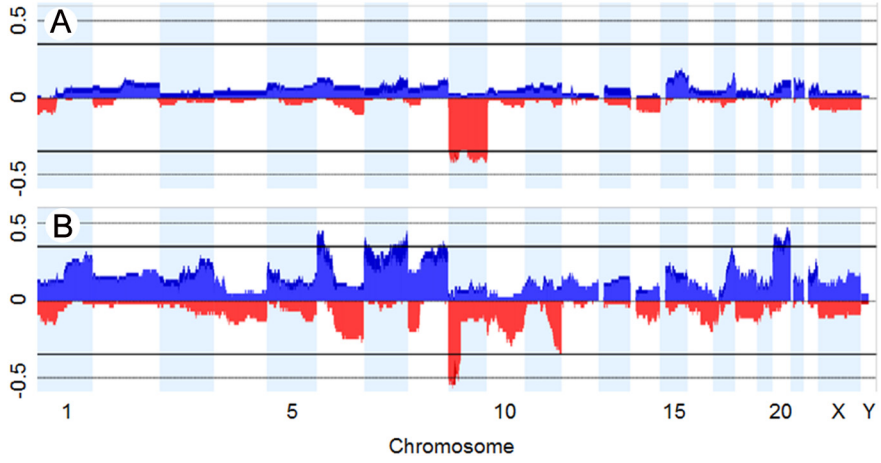

Fig. 4 Genomewide pattern of copy number gains and losses for borderline group (A, n = 63) and definitive melanomas (B, n = 44). Chromosomes are listed along the bottom; y-axis shows fraction of cohort exhibiting single copy gains (blue), high-level gains (≥4 copies total, dark blue), and heterozygous (red) and homozygous losses (dark red). Solid horizontal lines represent gain/loss in 35% of cases.

A representative case of definitive (nevoid) melanoma is presented in Fig. 1. It showed loss of p16 protein immunoexpression and corresponding homozygous loss of *CDKN2A* (9p21) on FISH and SNP array studies. The case is hence positive by SNP array, virtual FISH, and conventional FISH. The patient underwent wide local excision with negative sentinel lymph node biopsy. There was no evidence of disease recurrence or progression at a 24-month follow-up visit.

A representative case of a borderline melanocytic lesion involving the back of a 37-year-old woman with positive SNP array but negative virtual FISH is shown in Fig. 2. Several CNAs are present in this spitzoid lesion, consistent with aggressive behavior and leading to a diagnosis of favor spitzoid melanoma. Clinical follow-up is not available for the case.

A representative case with false-positive FISH is seen in Fig. 3, which depicts an atypical compound desmoplastic spitzoid melanocytic proliferation arising on the left elbow of a 31-year-old woman. There are gains and losses of entire chromosomes, which in the absence of focal abnormalities are interpreted as negative for melanoma and are instead more consistent with an indolent lesion [10]. By FISH, however, the presence of an additional copy of chromosome 11 led to a positive result by both conventional FISH and virtual FISH, which are hence both considered false positives. Clinical follow-up for this consult case was not available.

Compared to SNP array, virtual FISH failed to recognize 22 cases with concerning CNAs (Table 2, Fig. 2) and incorrectly classified as positive 3 cases with whole chromosome gains and losses (Table 2, Fig. 3). Agreement between the assays was 89% in the melanoma group but only 68% in the borderline group. Sensitivity of virtual FISH was 82% in the melanoma group but only 61% in the borderline group, using SNP array as the gold standard, whereas specificity was 100% and 84% in these groups, respectively.

A comparison of genomewide CNAs for borderline and definitive melanomas is shown in Fig. 4. CNAs are much more common in the melanoma group than in the borderline group, including more frequent gains and losses at the 5 FISH loci.

Virtual FISH results for each of the 5 probe loci are given in Table 3. A higher proportion of melanoma cases were positive for gain of *RREB1* and *MYC* and loss of *MYB* ($P < .05$ for all comparisons, χ^2) relative to borderline group. Differences in gains of *CCND1* and homozygous loss of *CKDN2A* did not reach significance.

There was close correlation between conventional and virtual FISH, with agreement in 19 of 22 virtual FISH–positive cases and 11 of 11 negative cases (total 30/33, 91%). All 3 discrepancies were cases found to be positive for homozygous *CDKN2A* loss by virtual FISH but not by conventional FISH (see "Discussion" for additional details).

4. Discussion

Our results show that SNP array is more sensitive than FISH in detecting potentially aggressive melanocytic lesions. Although the 2 techniques had a high level of agreement for definitive melanomas (89%), this was only 68% for the cohort of borderline lesions. The number of false negatives in the FISH group, using SNP array as the gold standard, rose from 5 of 44 (11%) in the melanoma group to 17 of 63 (27%) in the borderline group (Table 2), with a concordant drop in FISH sensitivity from 82% to 61%. This suggests that using FISH, rather than SNP array, as an ancillary tool in diagnosing borderline melanocytic lesions carries the risk of misclassifying many potentially aggressive lesions as benign, possibly leading to inadequate treatment.

The overall pattern of gains and losses across the genome in our cohort of definitive melanomas is very similar to that reported in earlier CGH studies of melanomas [7,8], with frequent gains of 1q, 6p, 7, 8q, 17q, and 20q, and losses of 6q, 9p, 10, and 11q (Fig. 4). A similar, albeit muted, pattern of gains and losses was found for the borderline cohort. One notable exception to this trend exists on chromosome 9, where loss of 9p, including the locus for *CDKN2A*, dominates in the melanoma group, whereas loss across the entire chromosome is common in the borderline group (Fig. 4). It is surprising that 9q is lost more frequently in the borderline group, both in absolute terms and relative to the frequency of 9p loss, and when compared not only to our own melanoma group but also to earlier melanoma CGH results [7,8]. Although loss of chromosome 9 has been suggested to occur early in the progression of melanoma [7] and hence might be expected to be commonly found in borderline lesions, these losses usually involve only the short arm of the chromosome [7,8]. Further study is needed to determine whether frequent loss of entire chromosome 9 is replicated in other cohorts of borderline melanocytic lesions, a result which would suggest that it may be a marker of intermediate malignant potential and/or an early stage of disease progression.

Prior studies have found that, among the set of FISH probes used, gain of 6p25 had the highest sensitivity in distinguishing

Table 3 Virtual FISH probe analysis for borderline lesions (n = 63) and definitive melanomas (n = 44)

Gene (locus)	Borderline lesions (%)	Melanomas (%)	*P*
RREB1 gain (6p25)	8 (13)	21 (48)	.00006
MYB loss (6q23)	7 (11)	12 (27)	.031
MYC gain (8q24)	6 (10)	14 (32)	.0036
CKDN2A homozygous loss (9p21)	11 (17)	13 (30)	.14
CCND1 gain (11q13)	6 (10)	5 (11)	.76

melanoma from nevi [18,19]. Similarly, in the present study, gain of 6p25 was the most common of the virtual FISH loci found to be aberrant in the melanoma group (present in 48% of cases, Table 3), while occurring only rarely in the borderline group (13%, $P = .00006$). In contrast to loss of 9p discussed above, gain of 6p may therefore typically occur late in disease progression. Additional studies are needed to determine whether this change portends a worse prognosis.

A recent study of 74 borderline melanocytic tumors was performed using the same set of FISH probes as used in our study save for 1 difference: a probe for *MYC* (8q24) was not included [20]. The authors found that only 10 cases (13%) were positive by FISH, a rate that is one-third of that found for virtual FISH in the current study (30/63, 48%). Only a small proportion of this discrepancy can be attributed to our inclusion of a probe for *MYC*—3 of the 30 FISH-positive borderline cases were solely positive at this locus. Hence, it is unclear why such different rates of FISH positivity were obtained between this earlier study and our own. Contributing to this discrepancy may have been differing thresholds for designating a lesion as "borderline" on histology; differences in FISH procedures and scoring cutoffs, for example, the earlier study used gain of 6p25 in >55% of nuclei to define positivity, whereas ours used gain in >29%; and a different distribution of lesion types, for example, only 21 of 73 (29%) of lesions in the previous study were in the Spitz family compared to 36 of 63 (57%) in the current study. Our use of virtual FISH may have also contributed to our higher positivity rate, as suggested by the finding of 3 of 33 cases (9%) to be positive by virtual but not conventional FISH. This results from the increased sensitivity of virtual FISH in identifying very focal deletions in *CDKN2A* (50-100 kb) that may be missed when using the 220-kb conventional FISH probe for this locus (unpublished data). In addition, it is possible that SNP array allows for confident calling of CNAs in a low fraction of the cells that would not meet the cutoff for FISH positivity. However, we did not directly observe this effect in our cohort.

Our results highlight the benefit of using SNP array over FISH in evaluating borderline lesions that are found to have gains and losses of entire chromosomes, consistent with indolent neoplasms. Three such cases were identified in our study —the spitzoid lesion in an adult presented in Fig. 3 and two pigmented nodules arising within giant congenital nevi in newborns. These latter lesions can be diagnostically challenging and often have worrisome features such as expansile growth, nuclear atypia, and high mitotic rate. Clinically, they may be rapidly growing and ulcerated, further raising the concern of malignancy. Although these lesions are typically benign proliferative nodules, which often regress spontaneously [21], congenital melanoma, or melanoma arising in a background of congenital nevus, is also known to occur in this setting [22-24]. Immunohistochemistry has little to no benefit in evaluating such lesions, further highlighting the value of SNP array as a diagnostic tool in assessing for malignancy. Gains and losses of entire chromosomes, which may lead to a (false) positive FISH result, are reassuring findings on SNP array and can help avoid misdiagnosis and overtreatment. One large CGH study found that only 5 of 122 (4%) melanomas demonstrated exclusively numerical abnormalities of entire chromosomes, with all 5 cases harboring losses of chromosomes 9 and/or 10 [10]. These losses were not seen in any of our 3 false-positive lesions.

Although the focus of the study was to compare SNP array to FISH in diagnosing melanocytic lesions, comparisons between the borderline and definitive melanoma groups were also possible. The borderline group tended to consist more often of younger female patients and the definitive melanoma group of older male patients. This is not unexpected given the association of increased age and male sex with melanoma [25]. Higher rates of SNP array and virtual FISH positivity were found in the melanoma group, as expected.

This study has some limitations. Clinical follow-up data were unfortunately unavailable for the majority of the cohort, as most definitive melanomas and nearly all borderline cases were referred in from external sites. Furthermore, many cases were quite recent, and hence, only very short follow-up periods would be currently available. Had clinical follow-up data been available for a large proportion of the cases, it would have allowed for categorization of borderline melanocytic lesions as ultimately behaving in a benign or malignant fashion. This could then have acted as a gold standard to which SNP array and FISH could both have been compared. Using instead the SNP array as gold standard to which FISH is compared presupposes that it has perfect sensitivity and specificity, which is known not to be the case even when distinguishing straightforward nevi and melanoma [8], let alone borderline lesions. Nevertheless, its genomewide scope provides a much more comprehensive view than FISH, and the approximation is thus felt to be reasonable. Another limitation of this study is its lack of power to stratify tumors by pigmentation (amelanotic versus pigmented); as melanogenesis can affect tumor behavior and clinical outcome [26,27], this type of subgroup analysis for borderline lesions could, if adequately powered, provide insight into genomic differences between these 2 groups.

The increased rate of positivity for SNP array relative to FISH raises the possibility that, rather than FISH suffering from low sensitivity, SNP array may instead have low specificity and "overdiagnose" melanoma. Although we cannot exclude this possibility until long-term follow-up data are collected, the association between increased CNAs and malignant behavior is well described [8,14,28-30], supporting our use of SNP array as the gold standard. This design is also supported by 2 studies which found borderline atypical Spitz tumors and spitzoid melanomas to be negative by FISH but harboring concerning CNA profiles on CGH and/or exhibiting metastatic spread [31,32]. Additionally, there are certain lesions where even genomewide array is insensitive at predicting aggressive behavior. For instance, deep penetrating nevus-like borderline tumors have been reported to exhibit malignant behavior despite having normal cytogenetic profiles by CGH [33]. Such reports suggest that a lack of sensitivity, rather than specificity, may be a greater problem when

diagnosing borderline lesions, casting in a favorable light the increased sensitivity of SNP array relative to FISH.

Many factors beyond sensitivity and specificity contribute to the decision of whether to use SNP array or FISH in diagnosing melanocytic lesions, including availability, cost, turnaround time, and lesion size/thickness, among others, with no "one size fits all" solution. Both techniques have their benefits and drawbacks, as reviewed elsewhere [34], which may recommend one over the other in certain circumstances.

Although FISH is highly effective in distinguishing between nevi and melanoma in cases where the histological diagnosis is relatively straightforward, it is not nearly as sensitive or specific as SNP array when applied to borderline melanocytic lesions. Our identification of several cases with concerning SNP array profiles but negative virtual and/or conventional FISH testing suggests that many patients with a borderline melanocytic lesion may require genomewide assessment of their lesion if they are to receive adequate treatment and reduce the risk of disease recurrence and progression.

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