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Nuclear and cytoplasmic features in the diagnosis of Clark's nevi

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Abstract

Background: Interpretation of Clark's nevi has generated debate over the years; although criteria have been proposed for grading morphological features of melanocytes, there is still confusion and variability in the assessment of these lesions.

Methods: This is a retrospective observational study conducted on 100 Clark's nevi and 84 melanomas. A single expert dermatopathologist evaluated all blinded and randomized photomicrographs of both the Clark's nevi and melanomas for the presence of 14 cytologic features. Subsequently, a multivariate model was used to obtain sensitivity and specificity.

Results: Clark's nevi showed a significantly higher frequency of absent-or-inconspicuous nucleoli over melanoma, whereas mitotic figures, pleomorphism, notching, multiple nucleoli, peppered moth nuclear pattern, flattened adjacent nuclei, prominent nucleoli and vesicular nucleus with rounded nucleoli were found significantly higher in frequency in melanomas.

Conclusion: Our data suggest that nuclear alterations are of value in the differentiation of atypical nevi from melanoma.

Keywords

chromatin; Clark's nevus; cytoplasm; dysplastic nevus; melanoma; nucleus

1 | INTRODUCTION

Clark's nevus (CN), also known as dysplastic nevus or atypical nevus, has generated a great amount of discussion over the past decades. The diagnosis is commonly based on architectural features, but the requirement for cytologic atypia is still debated and published criteria vary. In a previous study, we compared the cytological features of melanoma vs Spitz nevus, concluding that some features were of greater value than others.¹ Herein, we studied the cytological features of 100 CN and compared them with the 84 melanomas from this prior study. To our knowledge, there are no studies quantifying the presence of such features in CN in actual practice.

2 | METHODS

This is a retrospective observational study using high-powered photomicrographs rather than glass slides to maintain an element of blinding. CN cases were consecutively retrieved from the archives of the Medical University of South Carolina (MUSC) and University of California—Irvine (UCI), representing a period from 2012 to 2016. Institutional Review Boards (IRB) provided expedited review and approved the study in August 2016 for MUSC and exempted IRB review in September (UCI) (MUSC—Protocol 58621, UCI—Protocol 2016–3102). The original diagnoses were rendered by board-certified dermatopathologists from each institution and were confirmed by intradepartmental consultation and ancillary studies when required.

Cases from “special sites” including the scalp, face, breast, groin, hands and feet were excluded, as were combined nevi, dysplastic nevi with severe atypia (highly atypical nevi), and cases with inadequate material for definitive diagnosis. For CN cases, hematoxylin and eosin-stained slides were retrieved and high-resolution photomicrographs were obtained of each melanocytic lesion (Figure 1). Image capture settings (eg, Kohler illumination, resolution, image sensitivity, white balance, exposure, etc.) were standardized at each institution prior to collection. The fields with the most prominent abnormalities were captured. These images were stored in tagged image file format, deidentified, blinded, and randomly interspersed amongst similarly blinded melanoma cases. A single dermatopathologist then evaluated the photomicrographs for the presence of 14 cytologic features: mitotic figures, abnormal mitotic figures, estimated nuclear:cytoplasmic ratio greater than 4:1, pleomorphism with enlarged nuclei, nuclear molding, solid hyperchromasia, notching or corrugation of the nuclear envelope, flattening of adjacent nuclei, nuclear pseudoinclusions, vesicular nuclei with single rounded nucleoli, multiple nucleoli, absent or inconspicuous nucleoli, dusty cytoplasm and peppered moth chromatin pattern (Figures 2 and 3). Definitions of these features are summarized in Table 1. Except for mitotic figures and abnormal mitoses which were graded as all or none, cytologic features needed to be present in greater than 20% of nuclei in the field to be considered positive. The most atypical area of each lesion was assessed.

3 | STATISTICAL METHODS

Statistical analyses were performed using IBM SPSS advanced statistics version 23 (SPSS Inc., Chicago, Illinois). Categorical data were described in frequencies and percentages. Chi-square (χ^2) and Fisher's exact tests were used when appropriate to compare differences between groups of categorical data. Contingency tables were used with degrees of freedom $(r-1)(c-1)$. All P -values were 2-tailed. P -values of less than .05 were considered significant. A stepwise logistic regression model was generated to identify a set of variables that can best predict the outcome variable of interest. Sensitivity, specificity, and overall accuracy were computed for each step of the model. Pseudo R^2 were obtained using the "Cox and Snell" method with its "Nagelkerke" modification. The overall fitness of the model was also verified using "Hosmer-Lemeshow" test.

4 | RESULTS

The frequencies of the cytologic features studied are summarized in Table 2. The most frequent features in CNs were absent-or- inconspicuous nucleoli, estimated nuclear cytoplasmic ratio greater than 4:1, presence of solid hyperchromasia and to a lesser extent the presence of dusty cytoplasm.

Our data suggest that absent-or-inconspicuous nucleoli favor a diagnosis of CN over melanoma. (Figure 2) In contrast, mitotic figures, abnormal mitotic figures, pleomorphism with enlarged nuclei, notching, multiple nucleoli, peppered moth nuclear pattern, flattened adjacent nuclei, single prominent nucleoli and vesicular nucleus with single rounded nucleoli were all found at significantly higher frequency in melanomas (Table 3, Figure 3). Estimated high nuclear- cytoplasmic ratio (a feature often cited as a criterion) and solid nuclear hyperchromasia did not distinguish between the two.

For these significant cytologic features found in both CN and melanoma, the specificity and sensitivity of each feature to independently diagnose melanoma over CN were calculated and are summarized in Table 4.

By applying the regression model to the features noted above, for the diagnosis of melanoma, it was found that the combination of pleo- morphism with enlarged nucleus and notching had a specificity of 96%, sensitivity of 94% and overall accuracy of 95.1%, while the presence of mitotic figures together with pleomorphism and notching showed a specificity of 99%, sensitivity of 94% and overall accuracy of 96.7%. Adding other cytological features, although independently significant over CN, did not further improve this predictability in this model.

5 | DISCUSSION

Although we designed the methodology in order to avoid potential bias, several methodological and technical limitations remain for this study. For instance, while we took photographs in various magnifications, the photographer had to apply personal criteria when selecting those high-power fields that appeared to exhibit the most atypical features. Another potential limitation includes the lack of interobserver and intraobserver assessments. Our

study involves single-observer analysis given that non-controversial examples of melanoma and atypical nevus were assessed. Lastly, as unequivocal cases of melanoma and CN were utilized, significance of our findings in ambiguous cases is yet to be tested.

Several researchers have investigated the architectural and nuclear variations present in nevi and their association with those seen in melanoma.^{1,2} This legacy provided background to establish cytological criteria for CN.

Elder et al were the first to describe these features in the so-called dysplastic nevus syndrome; such features included: pleomorphism, hyperchromatism, dusty cytoplasm and prominent nucleoli.³ Rhodes et al subsequently, added others such as coarse granular chromatin and irregular nuclear borders.⁴

Arumi-Uria et al, in their study involving more than 6000 specimens diagnosed as CNs, categorized the cytological features according to the grade of atypia. Cytological features for mild atypia included collapsed cytoplasm, hyperchromatic, indented, condensed nuclei with no visible nucleolus or with very small one. Moderate atypia included enlarged hyperchromatic nuclei, small nucleolus and enlarged cytoplasm. Severe atypia included enlarged nucleus with large bizarre and hyperchromatic nuclei and dispersed chromatin and prominent nuclei.⁵

In our series, we observed more frequently the presence of features such as solid hyperchromasia (59 cases) and an increased nuclear cytoplasmic ratio (74 cases). These findings are similar to those described in the criteria of Arumi-Uria et al for cases with mild cytological atypia. Results revealed that, besides the presence of dusty cytoplasm, further degree of atypia was not frequently identified, at least in more than 20% of melanocytes per lesion in our cases. Features such as multiple nucleoli were not observed in any CN; in addition, single prominent nucleoli were present in only 1 case.

On the other hand, when pleomorphism with enlarged nucleus, notching and mitotic figures were present in more than 20% of melanocytes per lesion, altogether provided 99% specificity and 94% sensitivity to diagnose melanoma. When present in more than 20% of melanocytes per lesion, additional features, such as peppered moth nuclear pattern of chromatin and multiple nucleoli provide particular significance and might be helpful to identify melanomas. (Table 4).

The scope of our study was not to make recommendations. Nevertheless when cases show inconsistent cytological variations, architectural features should lead the logic of judgment.

6 | CONCLUSION

In practice, when evaluating CN, dermatopathologists take into consideration various factors including architecture, cytology, the clinical size of the nevus, extent of solar damage, demographics, and, whether or not the sample demonstrates the entire lesion. The importance of lesional architecture is undisputed. However, certain cytological features have diagnostic value, especially when evaluating melanoma.

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FIGURE 1. Clark's nevus. (A) Well-circumscribed melanocytic nevus with bridging formation. Note the absence of nucleoli, but presence of hyperchromasia. H&E $\times 200$

FIGURE 2.
Notched nuclear envelope, multiple nucleoli, flattening of adjacent nuclei, mitotic figure,
and peppered moth chromatin pattern (H&E $\times 1000$, digitally enlarged)

FIGURE 3.
Peppered moth wing courtesy of David Tomlinson PhD

TABLE 1.

Definitions of nuclear and cytoplasmic features evaluated in this study

Features	Definition
Mitotic figure	Condensed chromatin with irregular margins, representing a cell undergoing mitosis
Abnormal mitotic figures	Tripolar or bizarre shaped mitotic figures
Dusty cytoplasm	Granular appearing cytoplasm with gray- brown color
Inconspicuous nucleoli	Nucleoli that are not prominent
Molding	One nucleus indented by another adjacent nucleus
Flattening of adjacent nuclei	Grouping of nuclei with adjacent areas flattened
Notching	Sharply pointed nuclear membrane
Peppered moth nucleus	Clumped chromatin appearing as multiple dark dots within the nucleus.
pleomorphism with enlarged nuclei	Nuclei with variations in size (approximately more than 40 μm)
Pseudoinclusions	Invagination of cytoplasm into the nucleus seen as nuclear clearing
Solid hyperchromasia	Homogenous hyperchromatic nucleus with smooth contours
Vesicular nucleus with single rounded nucleoli	Nucleus displaying a prominent membrane with a pale/clear center
Multiple nucleoli	Two or more visible nucleoli

TABLE 2.

Frequencies of cytological features in melanomas and Clark's nevi

Cytological feature	Melanoma (n, %)	Clark's nevi (n = %)
Mitotic figures	33 (39)	0
Abnormal mitotic figures	20 (24)	0
Dusty cytoplasm	26 (31)	27
Absent/inconspicuous nucleoli	56 (67)	94
Molding	36 (43)	2
Multiple nucleoli	62 (74)	0
Notching/corrugation of nuclear envelop	72 (86)	1
Peppered moth nucleus	62 (74)	0
Pleomorphism with enlarged nuclei	78 (93)	3
Single prominent nucleoli	17 (20)	5
Pseudoinclusions	16 (19)	18
Solid hyperchromasia	60 (71)	59
Vesicular nucleus with single rounded nucleolus	6(7)	0
Estimated N/C ^a ratio > 4:1	57 (68)	74

^aN/C ratio: nuclear cytoplasmic ratio.

TABLE 3.

Cytological features helping the diagnosis of melanomas over Clark's nevi

Cytological feature	Melanoma (<i>n</i> = 84) (%)	Clark's nevi (<i>n</i> = 100) (%)	Chi-square	<i>P</i> -value
Pleomorphism with enlarged nuclei	93	3	149.5	<.001
Notching	86	1	136.8	<.001
Multiple nucleoli	74	0	111.3	<.001
Peppered moth nuclear pattern	74	0	111.3	<.001
Flattening of adjacent nuclei	91	26	76.8	<.001
Mitotic figures	39	0	47.8	<.001
Molding	43	2	46.5	<.001
Abnormal mitotic figures	24	0	26.7	<.001
Absent/ inconspicuous nucleoli	67	94	22.6	<.001
Single prominent nucleolus	20	5	10.07	.002
Vesicular nuclei with single rounded nucleolus	7	0	Fisher's exact	.008

TABLE 4.

Sensitivity, specificity and overall accuracy of cytological features found to help diagnosing melanoma over Clark's nevi

Cytological feature	Specificity (%)	Sensitivity (%)	Overall accuracy (%)
Pleomorphism with enlarged nuclei	97	92.9	95.1
Notching	99	85.7	92.9
Multiple nucleoli	100	73.8	88
Peppered moth nuclear pattern	100	73.8	88
Flattening of adjacent Nuclei	74	90.5	81.5
Mitotic figures	100	39.3	72.2
Molding	98	42.9	72.8
Abnormal mitotic figures	100	23.8	65.2
Single prominent nucleolus	95	20.2	60.8
Vesicular nuclei with single rounded nucleolus	100	7.1	57.6