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The self-reported use of immunostains and cytogenetic testing in the diagnosis of melanoma by practicing U.S. pathologists of 10 selected states

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Abstract

Background—The diagnosis of melanoma can be challenging, especially in lesions for which the histopathologic criteria bridge two or more taxonomic categories. Newer genomic analytical methods of fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) have been introduced as ancillary techniques to differentiate benign and malignant melanocytic proliferations.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Self-reported practice patterns and attitudes of pathologists who use or do not use fluorescence *in situ* hybridization and comparative genomic hybridization for evaluation of melanocytic lesions

Methods—We evaluated how pathologists perceive and are incorporating these new cytogenetic testing technologies into their practices. We conducted a study of 207 U.S. pathologists who interpret melanocytic lesions in clinical practice in 10 SEER states. Pathologists were surveyed regarding perceptions and utilization of FISH and/or CGH in their clinical practices.

Results—Results showed that 38% of pathologists use FISH and/or CGH in interpreting melanocytic lesions. Pathologists reporting FISH and/or CGH use were significantly younger ($p < 0.05$), were fellowship trained or board certified in dermatopathology ($p < 0.001$) and were affiliated with an academic institute ($p < 0.001$). Pathologists reporting that their colleagues consider them an expert in the assessment of melanocytic lesions were more likely to employ FISH and/or CGH in their practices than non-experts.

Conclusions—Early users of cytogenetic testing technologies in cutaneous pathology are more likely to be younger, affiliated with an academic institution and fellowship trained or board certified in dermatopathology.

Keywords

CGH; dermatopathology; FISH

The histopathologic diagnosis of melanocytic lesions is often difficult because of ambiguities and subjectivity in arriving at diagnostic inferences from the criteria-based methodology. Newer genomic analytical methods of fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) have been introduced as ancillary techniques to help differentiate between benign and malignant melanocytic proliferations.¹ CGH and FISH do not require fresh tissue and can be performed on tissue prepared for permanent sections. FISH, which targets specific DNA sequences *in situ*, can be used in heterogeneous tissue with small amounts of tumor cells. CGH assesses the entire genome for changes in DNA copy number, with the goal of differentiating melanoma from benign melanocytic lesions. CGH does require adequate tissue, as copy number changes in chromosomes can only be detected if the abnormality is present in at least 30–50% of the cells. Both FISH and CGH tests categorize lesions based on specific underlying cytogenetic aberrations, thereby aiming to advance disease prognostication beyond that achieved by conventional histopathology and immunohistochemistry.¹

Although these new technologies are available, the ways in which pathologists perceive and incorporate new technology into their practices are inadequately understood. Diffusion of new medical technology can take 15 or 20 years to fully reach widespread adoption in clinical practice, with extensive variability among practices and practitioners. We report results of a survey of U.S. pathologists regarding their perceptions and utilization of FISH and/or CGH in their own clinical practices, and we describe the characteristics of the early users of the technologies.

Methods

The details of the melanoma pathology study (M-Path) are summarized in detail elsewhere.^{2,3} In brief, pathologists who interpret melanocytic lesions as part of their clinical practice were recruited to participate in an online survey and test-set interpretation. Approval

was obtained from the Institutional Review Board of the University of Washington Human Subjects Division. From July 2013 to April 2014, 864 community and academic pathologists from 10 geographically diverse Surveillance, Epidemiology and End Results (SEER) states (California, Connecticut, Iowa, Kentucky, Louisiana, Hawaii, New Jersey, New Mexico, Utah and Washington) were invited via e-mail, postal mail and telephone calls to participate. To be eligible, pathologists must have completed medical school and residency training, have actively interpreted melanocytic skin biopsies within the previous year and have planned to continue interpreting melanocytic skin lesions for the subsequent 2 years. The online survey included questions on general demographics, clinical experience and frequency of immunohistochemistry and FISH and/or CGH cytogenetic testing in their clinical practice. Participants were asked 'In what circumstances do you request FISH/CGH or other molecular analysis?' and 'In what circumstances do you request immunohistochemistry?' Participants' responses to both questions were collapsed into two categories: 'I do not use' vs. all other responses. The full survey is available online.⁴

Study results for participants are analyzed using descriptive statistics. Categorical variables are tabulated as frequencies and percentages and continuous variables are summarized as mean $\pm$ SD. Associations between participant categorical responses and their reported use of FISH and/or CGH were assessed using the Chi-square statistic (Fisher's exact when cells have expected counts of <5) and for ordered categories the Mantel Haenszel Chi-square test for trend. The *t*-test was used to compare differences between means of continuous measures across the participants' reported use or non-use of FISH and/or CGH. *p*-values were two-sided and evaluated using a two-sided significance level of 0.05. All statistical analyses were performed using SAS for Windows v9.4 (SAS, Inc., Cary, NC, USA).

Results

Of the 301 eligible responding pathologists, 207 completed the survey (response rate 69%). Characteristics of pathologists by their reported use of FISH and/or CGH are shown in Table 1. The average age of the participants was 51 years, and the majority of respondents were males ($n = 123$; 59%), not affiliated with a medical center ($n = 148$; 71%) and practiced in California ($n = 118$; 57%).

Thirty-eight percent (79/207) of participants reported using FISH and/or CGH in their practices, with 19% (15/79) using FISH and/or CGH tests to improve accuracy and 76% (60/79) reporting that these tests help settle ambiguous cases. As a comparison, the majority of participants (95%; 197/207) reported use of immunohistochemistry for some of their melanocytic lesions. Regarding the purpose of employing immunohistochemistry, 42% (83/197) stated that they use it to improve the accuracy of melanoma diagnosis, and 50% (99/197) reported that it helps to settle an ambiguous case.

The average ages of pathologists who use vs. do not use FISH and/or CGH are 49 and 52 years, respectively ($p < 0.05$). Pathologists utilizing FISH and/or CGH were more likely to be fellowship trained or board certified in dermatopathology, affiliated with an academic medical center and have higher volume clinical practices (Table 1). In addition, regional

variation was noted in reported use of FISH and/or CGH, such that pathologists practicing in the San Francisco Bay Area reported the highest rates.

Moreover, pathologists reporting that their colleagues consider them an expert in the assessment of melanocytic lesions were more likely to employ FISH and/or CGH in their practices than non-experts. In general, participants who reported using FISH and/or CGH tests also reported that they were confident in interpreting melanocytic lesions and that they found interpreting melanocytic lesions enjoyable. All differences were statistically significant (Table 1).

There were no statistically significant differences between users and non-users of FISH and/or CGH with respect to gender, years interpreting melanocytic lesions, number of melanocytic lesions per month for which they request a second opinion, portion of melanocytic lesions that they diagnose as borderline when interpreting melanocytic lesions compared to other types of pathology, or opinion on how challenging melanocytic lesions are to interpret (see Table 1 and Table S1, Supporting Information).

Discussion

Our study evaluated the characteristics of U.S. pathologists who are early adopters of cytogenetic testing for evaluating melanocytic lesions in their clinical practices. Thirty-eight percent of respondent pathologists report utilizing FISH and/or CGH; these pathologists are more often affiliated with academia and work in specific geographic regions compared with non-users of FISH and/or CGH.

As is often the case with new medical technology, the sensitivity and specificity of FISH and CGH in the diagnosis of melanoma in clinical practice are still controversial. In a large study, Gerami et al. analyzed 322 tumors, including 152 melanomas and 170 nevi, using four-probe FISH assay targeting 6p25, 6q23, Cep6 and 11q13. The sensitivity and specificity of FISH assay were 86.7 and 95.4%, respectively.⁵ Another research group evaluated 90 ambiguous melanocytic lesions with interpretable FISH; by comparison with clinical outcome, the sensitivity and specificity of histopathologic review were 95 and 52%, while the sensitivity and specificity of FISH were 43 and 80%.⁶ In a previous study of 132 melanomas vs. 54 non-melanoma melanocytic lesions, 96% of melanomas had chromosomal alterations found through CGH compared with 13% of non-melanoma melanocytic lesions, and the non-melanoma melanocytic lesions with CGH aberrations were all diagnosed as Spitz nevi; other lesions, such as benign nevi, congenital nevi and blue nevi did not have any chromosomal irregularities on CGH.⁷ In other studies, CGH has shown a lack of chromosomal abnormalities when examining benign nevi and ‘borderline’ lesions, such as congenital nevi with exuberant basilar proliferation simulating superficial spreading melanoma *in situ*. Most of the studies of CGH and FISH have evaluated these tests against lesions diagnosed by expert opinion, with or without consensus panels. In one study, Gerami et al. identified three fatal lesions in a study set of 76 problematical Spitzoid lesions using a FISH probe set that included 9p21, the locus of the p16 tumor suppressor gene.⁸

Although FISH, CGH and other cytogenetic tests can potentially assist with the diagnosis of ambiguous melanocytic lesions, there are still false positive and false negative results. We noted that pathologists who were considered experts in melanocytic lesions, reviewed a higher volume of melanocytic cases or who received more consult cases are more likely to use FISH and/or CGH than other pathologists. It is possible that those pathologists interpret more difficult and borderline cases than average participants, leading to enhanced utilization of FISH and/or CGH to arrive at a more confident diagnosis.

FISH and/or CGH require specialized expertise to perform the tests and analyze the results and as a result are only available at certain laboratories in the United States. The disproportionate amount of pathologists utilizing these technologies who live in the San Francisco Bay Area may reflect other factors not assessed in our survey, such as accessibility of laboratories providing FISH and/or CGH, insurance issues and/or reimbursement practices in different health systems and regions of the country.

The cost of FISH or CGH may limit the spread of these new technologies. Through an informal survey of 10 laboratories in the United States, we found that charges range from \$1000 to 2000 for FISH and \$1300 to 6000 for CGH per skin biopsy. Furthermore, performance of FISH and CGH on melanocytic lesions is variably covered by insurance payers, with reimbursement varying by the insurance company. There are currently no FDA guidelines or regulations on how these tests should be performed and interpreted; they are investigational techniques and are not approved by the FDA for diagnosing melanoma. More long-term follow-up studies are needed to confirm the diagnostic value of FISH and CGH before they are widely accepted as ancillary techniques for the diagnosis of melanoma.

Studies in economics, sociology and applied mathematics establish that early adopters play an important role in the diffusion of new technologies.⁹ The diffusion of new technology in medicine is thought to be influenced by knowledge dissemination, adoption by early users and policies and rules promulgated by regulatory agencies. The perceptions of FISH and CGH that early adopters identified in this study may impact the general population of physicians' adoption and usage of FISH and CGH over time.

The limitations of our study include potential bias from self-reporting, participation limited to pathologists from only 10 states (mostly from California), and a lack of distinction between FISH, CGH and other cytogenetic tests on the survey itself. While the data highlight regional variation in diffusion of medical technology, it is unknown if the findings are translatable to the remainder of the United States. Additionally, this study describes self-reported use of early adoption and it will be important in future studies to obtain data on the impact of these tests on clinical practice and patient outcomes.

In summary, our survey of U.S. pathologists in 10 states regarding their perceptions and utilization of cytogenetic testing in their clinical practices indicates that 38% of pathologists report employing FISH and/or CGH in interpreting melanocytic lesions. These pathologists are more likely to have fellowship training or board certification in dermatopathology, affiliation with an academic medical center, more experience in interpreting melanocytic

lesions and be from specific regions in the United States with strong academic centers offering testing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Self-reported demographic and practice patterns of pathologists who use or do not use fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization (CGH) for evaluation of melanocytic lesions.

	Total N (%)	Use FISH/CGH n(%)	Do not use FISH/CGH n(%)	p-Value
Participant characteristics	207 (100)	79 (38)	128 (62)	
Demographics				
Age				
30–39	36 (17)	21 (58)	15 (42)	0.017
40–49	59 (29)	20 (34)	39 (66)	
50–59	71 (34)	27 (38)	44 (62)	
60	41 (20)	11 (27)	30 (73)	
Sex				
Male	123 (59)	52 (42)	71 (58)	0.14
Female	84 (41)	27 (32)	57 (68)	
Fellowship training or board certification				
Dermatopathology	81 (39)	57 (70)	24 (30)	<0.001
Other	126 (61)	22 (17)	104 (83)	
Affiliation with an academic medical center				
Yes, primary position	21 (10)	17 (81)	4 (19)	<0.001
Yes, adjunct/affiliated clinical faculty	38 (18)	21 (55)	17 (45)	
No	148 (71)	41 (28)	107 (72)	
Practice location				
California				
The San Francisco Bay Area*	33 (16)	21 (64)	12 (36)	0.004
All other counties	85 (41)	26 (31)	59 (69)	
All other states	89 (43)	32 (36)	57 (64)	
Clinical confidence and enjoyment of clinical work				
In general, how confident are you in your assessments of melanocytic skin lesions?				
Extremely confident	35 (17)	21 (60)	14 (40)	<0.001
Very confident	97 (47)	39 (40)	58 (60)	
Moderately confident	46 (22)	11 (24)	35 (76)	
Somewhat confident	16 (8)	5 (31)	11 (69)	
Rarely confident	13 (6)	3 (23)	10 (77)	
Not at all confident	0 (0)	0	0	
Interpreting melanocytic skin lesions is enjoyable				
Strongly agree	17 (8)	13 (76)	4 (24)	0.002
Agree	76 (37)	31 (41)	45 (59)	
Slightly agree	51 (25)	18 (35)	33 (65)	
Slightly disagree	36 (17)	9 (25)	27 (75)	
Disagree	18 (9)	7 (39)	11 (61)	
Strongly disagree	9 (4)	1 (11)	8 (89)	
Clinical experience				

	Total N (%)	Use FISH/CGH n(%)	Do not use FISH/CGH n(%)	p-Value
Benign melanocytic lesions interpreted per month				
Mean ± SD	116 ± 170	198 ± 230	66 ± 88	<0.001
Melanoma interpreted per month				
Mean ± SD	9 ± 12	15 ± 16	5 ± 6	<0.001
Number of melanocytic lesions received as a second opinion consult per month				
Mean ± SD	13 ± 19	22 ± 25	8 ± 11	<0.001
Number of melanocytic lesions requested as a second opinion consult per month				
Mean ± SD	5 ± 7	5 ± 5	6 ± 8	0.26
Do your colleagues consider you an expert in the assessment of melanocytic lesions?				
Yes	88	56 (64)	32 (36)	<0.001
No	119	23 (19)	96 (81)	

Except where otherwise noted, data are presented in frequencies and percentages. Percentages are presented by column total or by row group.

* Includes Placer and Sacramento counties in addition to the nine Bay Area counties (Alameda, Contra Costa, Marin, Napa, San Francisco, San Mateo, Santa Clara, Solano and Sonoma).