Published in final edited form as:

Am J Surg Pathol. 2011 August ; 35(8): 1146–1150. doi:10.1097/PAS.0b013e318222a634.

Assessment of Copy Number Status of Chromosomes 6 and 11 by FISH Provides Independent Prognostic Information in Primary Melanoma

Jeffrey P. North, MD^{1,2}, John T. Vetto, MD³, Rajmohan Murali, MBBS, MD⁴, Kevin P. White, MD¹, Clifton R. White Jr., MD¹, and Boris C. Bastian, MD^{2,4}

¹Oregon Health & Science University, Portland, OR. Department of Dermatology

²University of California, San Francisco, San Francisco, CA. Department of Dermatology and Pathology

³Oregon Health & Science University, Portland, OR. Division of Surgical Oncology, Department of Surgery

⁴Memorial Sloan-Kettering Cancer Center, New York

Abstract

Melanoma incidence has been rising steadily for decades, while mortality rates have remained flat. This type of discordant pattern between incidence and mortality has been linked to diagnostic drift in cancers of the thyroid, breast, and prostate. Ancillary tests such as fluorescent in situ hybridization (FISH) are now being used to help differentiate melanomas from melanocytic nevi. Multicolor FISH has been shown to distinguish between these two with 86.7% sensitivity and 95.4% specificity. To assess the ability of FISH to differentiate melanomas with metastatic or lethal potential from those with an indolent disease course, we performed FISH with probes targeting 6p25, centromere 6, 6q23, and 11q13 on 144 primary melanomas with a minimal tumor thickness of 2 mm and compared the development of metastatic disease and melanoma-specific mortality as well as relapse-free and disease-specific survival between FISH-positive and negative cases. 82% of melanomas were positive by FISH according to previously defined criteria. The percentage was significantly higher (93%) in cases that developed systemic metastases (n=43) than in patients that did not (77%, n=101). FISH-positive primaries had a significantly increased risk of metastasis or melanoma-related death compared to FISH-negative cases (odds ratio 4.11, confidence interval (CI) 1.14-22.7 and 7.0, CI 1.03-300.4, respectively). FISH status remained an independent parameter when controlling for known prognostic factors. This data indicates that the group of melanomas diagnosed with routine histopathology that lack aberrations detected by FISH is enriched for melanomas with a more indolent disease course. This suggests that molecular techniques can assist in a more accurate identification of tumors with metastatic potential.

Corresponding Author: Boris C. Bastian, M.D., Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, 212-639-8410, 212-772-8521 fax, bastianb@mskcc.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Histopathologic assessment is the gold standard for the diagnosis of melanoma and allows for an unequivocal diagnosis in the majority of cases. However, there is a subset of melanocytic neoplasms which cannot be reliably classified as benign or malignant using routine histologic evaluation. Multiple studies have shown diagnostic discordance rates for melanocytic neoplasms evaluated by routine methods ranging from 14-38% even among expert dermatopathologists^{4,7,13,15,18,19,20}.

Over the past few decades the incidence of melanoma has increased continuously, while melanoma mortality rates have remained comparably constant²¹. This dichotomy is difficult to reconcile considering that no advances in melanoma therapy showing significant mortality benefit were made during this time period. Advances in melanoma treatment with BRAF inhibition and anti-CTLA4 therapy^{8,12} have occurred too recently to be counterbalancing factors. In addition, during this same time period it has been noted that the increase in the diagnosis of melanoma parallels an increase in the number of skin biopsies²². A similar pattern has been observed in cancers of the thyroid, breast, and prostate, where incidences have also paralleled the number of diagnostic studies²¹. While it can be argued that the lack of increase in mortality is due to removal of cancers at an earlier stage thus preventing their progression to a lethal stage, this effect would result in a drop in mortality in subsequent years. Such an effect has not occurred. Instead the data suggest that the increase in incidence is due to an increase of cases that are biologically indolent, i.e. do not have metastatic potential. Diagnostic drift, a change in diagnostic criteria that results in classification of tumors as malignant that were previously classified as benign, is a likely contributing factor to this phenomenon¹⁴.

Diagnostic drift leading to melanoma overdiagnosis is a significant problem as it leads to significant morbidity through unnecessary surgical and medical treatment, psychological trauma, and inflated health care costs. We have recently described a panel of FISH probes which detects common chromosomal imbalances in melanoma and distinguishes benign and malignant melanocytic tumors with 86.7% sensitivity and 95.4% specificity independent of histomorphology⁹. The diagnostic capability of this test in distinguishing nevi from melanomas has been validated in additional studies^{2,3,6,10,11,16,17}. In this study we analyze a cohort of unequivocal melanomas to determine whether clonal chromosomal imbalances detectable by FISH provide independent information that can identify tumors with metastatic potential.

Methods

We retrieved paraffin-embedded tissue blocks of primary melanomas diagnosed prior to December 2007 measuring at least 2 mm in depth from the archives of the OHSU Dermatopathology Section and Pathology Department. All cases had an unequivocal pathologic diagnosis of melanoma and were independently reviewed in blinded fashion by a second dermatopathologist (RM). All studies were approved by the institutional review boards at OHSU and UCSF. FISH analysis with probes targeting 6p25 (RREB1), centromere 6, 6q23 (MYB), and 11q13 (CCND1) was carried out as previously described⁹.

Signals were scored according to previously determined criteria and thresholds⁹ and considered positive if 55% or more nuclei had higher 6p25 signal counts than Cep6 signal counts (6p imbalance), if 40% of nuclei had lower 6q23 signal counts than Cep6 (6q imbalance), if 29% or more nuclei had more than two signals for 6p25 (6p gain), or if 38% or more nuclei had more than two signals for 11q13 (11q gain). Clinical follow up data was obtained from the OHSU tumor registry, medical record and sentinel lymph node database.

Statistical Analysis

Fisher's exact test and the Kaplan-Meier estimator were used to analyze FISH results in relation to disease-specific survival (DSS) as well as disease-free survival (DFS). Fisher's exact test and Wilcoxon's rank sum test were used to test for significant associations between FISH results and established prognostic factors in melanoma. A Cox's proportional hazard model was used for multivariate analysis.

Results

144 melanomas were successfully analyzed by FISH, 118 (82%) of which tested positive by at least one of the 4 established criteria⁹. The pertinent clinical data and breakdown of melanoma subtypes included in the study are depicted in tables 1 and 2 respectively. Patients whose primary tumor tested positive tended to be older than patients with FISH-negative primaries ($p=0.049$). No association between FISH status and other established prognostic factors such as gender, ulceration, tumor thickness, sentinel node status, and anatomic location was found (Table 1). The median follow-up time was 32 months. A total of 43 (30%) cases had metastasized by the time of last follow-up, 40 (93%) of which tested positive by FISH. 27 patients died of melanoma with all except one (96.7%) exhibiting chromosomal aberrations detected by FISH. By contrast, only 77% of patients that did not meet one of these two endpoints were FISH-positive. These prognostic differences between FISH-negative and FISH-positive tumors were statistically significant ($p=0.02$ for disease-specific survival and $p=0.04$ for disease-free survival). Three cases with stage IV (all IVc) disease were negative by FISH, one of which died from metastatic melanoma. Interestingly, the other two stage IV melanomas that were FISH-negative had a complete response to interleukin-2 (IL-2) therapy with no evidence of disease at 32 and 34 months of follow up.

Patients whose primary melanoma tested positive for FISH had a significantly increased risk for the development of metastatic disease (OR 4.11, $p=0.02$, CI 1.14-22.7) or death from melanoma (OR 7.0, $p=0.04$ CI 1.03-300.4) compared to FISH-negative cases. Patients with FISH-positive primaries also showed a decrease in disease-specific survival and disease-free survival by Kaplan-Meier analysis (figures 1 and 2, respectively) compared to FISH-negative cases. In a multivariate analysis controlling for sentinel lymph node status, tumor thickness, ulceration, and patient age, a positive FISH result was independently associated with increased risk for metastasis (HR 5.9 $p=0.04$, CI 1.06-32.44). The trend was similar for independently predicting death from melanoma, but fell short of statistical significance (HR 5.7 $p=0.11$, CI 0.67-48.8).

We tested whether adjustment of the thresholds for signal counts of the four FISH probes could improve the discrimination between metastatic and non-metastatic cases and

determined the optimal combinations to distinguish metastatic tumors in this set of 144 melanomas (Table 3).

Discussion

Similar to prior studies, FISH analysis with probes targeting chromosomes 6 and 11 is negative in a minority of primary melanomas (18% in this study compared to 0-25% in other studies^{2,3,6,9-11,16,17}). Several reasons may account for a negative FISH test result in histopathologically diagnosed melanomas. Not all melanomas have aberrations involving these two chromosomes^{1,5}, and it is therefore expected that the current FISH test misses such melanomas. This supposition is supported by the observation that the prevalence of aberrations involving the chromosomes enumerated by the assay can vary among biological variants of melanoma. For example, amplification of chromosome 11q13 is more common in melanomas arising on acral skin and skin with chronic sun-induced damage⁵. As shown in table 2, our study shows a distribution of subtypes similar to what would be expected for most US practices and therefore includes a genetically diverse group of tumors.

Negative results will also be expected in melanocytic neoplasms that histopathologically simulate melanoma without having the malignant biologic potential of a bona fide melanoma. The category of spitzoid melanocytic neoplasia is notoriously enriched for these cases due the lack of unequivocal histopathological criteria for the distinction of spitzoid melanoma and neoplasms within the range of “atypical Spitz tumors”. Our study of 144 melanomas included six spitzoid melanomas (Table 2). Independent histopathologic review confirmed the diagnosis of melanoma in all 144 cases with the exception of one of these 6 spitzoid melanomas, in which the possibility of an “atypical Spitz tumor” was raised (Figure 3). The statistical results remained unaffected when this case was excluded from the analysis (risk for metastasis: OR 5.73, $p=0.046$, CI-1.03-31.97 for univariate and HR 4.51, $p=0.039$, CI-1.08-18.77 for multivariate analysis; melanoma-specific mortality: OR-5.66, $p=0.042$, CI-0.71-45.28). Our findings raise the possibility that, in addition to the spitzoid group of melanocytic neoplasms, other types of melanocytic tumors that may appear to be melanoma by conventional histopathologic methods do not possess metastatic potential²¹, and that the absence of chromosomal aberrations may provide an opportunity to identify them.

In this study, primary melanomas without chromosomal aberrations detectable by FISH for chromosomes 6 and 11 were significantly less likely to metastasize and cause death. The prognostic information of a positive FISH test was independent of sentinel lymph node status, tumor thickness, ulceration, and patient age, verifying that FISH detects unique prognostic features in melanoma. Our results therefore indicate that the approximately 13-25% of melanomas that test negative by FISH contain a higher proportion of cases with lower metastatic risk, or conversely, that the assay's sensitivity for melanomas with metastatic potential is greater than for melanomas as a whole. We also found that the ability to predict future metastasis or disease-specific survival could be improved by modifying the previously established thresholds determined for assisting in the diagnostic distinction between nevi and melanoma. However, these thresholds will need to be independently tested in separate cohorts of melanoma to verify their validity.

Limitations in this study include incomplete follow-up data in some cases. Due to the nature of our tertiary care center, some patients were only seen for sentinel lymph node biopsy with limited follow up thereafter. Also, we only tested melanomas with a minimum thickness of 2 mm to maximize the number of primary endpoints reached. While it is expected that our results would apply to thinner melanomas, future studies are needed to verify this.

In summary, we found significant differences in frequency of metastasis and disease-specific survival between FISH-positive and FISH-negative cases. The FISH test result yielded prognostic information predicting metastasis independent of tumor thickness and sentinel lymph node status. Our results thus suggest that a positive FISH test not only distinguishes melanomas from nevi with high specificity as previously shown, but also identifies melanomas with higher risk of metastasis. Conversely, while a negative FISH test cannot exclude a diagnosis of melanoma, it decreases the likelihood of one with metastatic potential. Future studies to confirm these results and explore additional FISH probes of potential prognostic value can increase the utility of FISH in detecting melanomas with metastatic potential and thereby identify patients who would benefit from more aggressive treatment and monitoring plans.

Acknowledgments

Supported by a grant from the National Institutes of Health (RO1CA1315241) to BCB.

References

1. Bastian BC, Olshen AB, et al. Classifying melanocytic tumors based on DNA copy number changes. *Am J Pathol.* 2003; 163(5):1765–1770. [PubMed: 14578177]
2. Busam KJ, Fang Y, et al. Distinction of conjunctival melanocytic nevi from melanomas by fluorescence in situ hybridization. *J Cutan Pathol.* 2010; 37(2):196–203. [PubMed: 20100177]
3. Clemente C, Bettio D, et al. A fluorescence in situ hybridization (FISH) procedure to assist in differentiating benign from malignant melanocytic lesions. *Pathologica.* 2009; 101(5):169–174. [PubMed: 20218056]
4. Corona R, Mele A, et al. Interobserver variability on the histopathologic diagnosis of cutaneous melanoma and other pigmented skin lesions. *J Clin Oncol.* 1996; 14(4):1218–1223. [PubMed: 8648377]
5. Curtin JA, Fridlyand J, et al. Distinct sets of genetic alterations in melanoma. *N Engl J Med.* 2005; 353(20):2135–2147. [PubMed: 16291983]
6. Dalton SR, Gerami P, et al. Use of fluorescence in situ hybridization (FISH) to distinguish intranodal nevus from metastatic melanoma. *Am J Surg Pathol.* 2010; 34(2):231–237. [PubMed: 20087158]
7. Farmer ER, Gonin R, et al. Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. *Hum Pathol.* 1996; 27(6):528–531. [PubMed: 8666360]
8. Flaherty KT, Puzanov I, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med.* 2010; 363(9):809–819. [PubMed: 20818844]
9. Gerami P, Jewell SS, et al. Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. *Am J Surg Pathol.* 2009; 33(8):1146–1156. [PubMed: 19561450]
10. Gerami P, Mafee M, et al. Sensitivity of fluorescence in situ hybridization for melanoma diagnosis using RREB1, MYB, Cep6, and 11q13 probes in melanoma subtypes. *Arch Dermatol.* 2010; 146(3):273–278. [PubMed: 20231497]
11. Gerami P, Wass A, et al. Fluorescence in situ hybridization for distinguishing nevoid melanomas from mitotically active nevi. *Am J Surg Pathol.* 2009; 33(12):1783–1788. [PubMed: 19809275]

12. Hodi FS, O'Day SJ, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363(8):711–723. [PubMed: 20525992]
13. Kempf W, Haeffner AC, et al. Experts and gold standards in dermatopathology: qualitative and quantitative analysis of the self-assessment slide seminar at the 17th colloquium of the International Society of Dermatopathology. *Am J Dermatopathol*. 1998; 20(5):478–482. [PubMed: 9790109]
14. Levell NJ, Beattie CC, et al. Melanoma epidemic: a midsummer night's dream? *Br J Dermatol*. 2009; 161(3):630–634. [PubMed: 19519827]
15. Lodha S, Sagar S, et al. Discordance in the histopathologic diagnosis of difficult melanocytic neoplasms in the clinical setting. *J Cutan Pathol*. 2008; 35(4):349–352. [PubMed: 18333894]
16. Morey AL, Murali R, et al. Diagnosis of cutaneous melanocytic tumours by four-colour fluorescence in situ hybridisation. *Pathology*. 2009; 41(4):383–387. [PubMed: 19404853]
17. Newman MD, Lertsburapa T, et al. Fluorescence in situ hybridization as a tool for microstaging in malignant melanoma. *Mod Pathol*. 2009; 22(8):989–995. [PubMed: 19448594]
18. Santillan AA, Messina JL, et al. Pathology review of thin melanoma and melanoma in situ in a multidisciplinary melanoma clinic: impact on treatment decisions. *J Clin Oncol*. 2010; 28(3):481–486. [PubMed: 20008627]
19. Shoo BA, Sagebiel RW, et al. Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. *J Am Acad Dermatol*. 2010; 62(5):751–756. [PubMed: 20303612]
20. Veenhuizen KC, De Wit PE, et al. Quality assessment by expert opinion in melanoma pathology: experience of the pathology panel of the Dutch Melanoma Working Party. *J Pathol*. 1997; 182(3): 266–272. [PubMed: 9349228]
21. Welch HG, Black WC. Overdiagnosis in cancer. *J Natl Cancer Inst*. 2010; 102(9):605–613. [PubMed: 20413742]
22. Welch HG, Woloshin S, et al. Skin biopsy rates and incidence of melanoma: population based ecological study. *BMJ*. 2005; 331(7515):481. [PubMed: 16081427]

Figure 1. Disease-Specific Survival, $p=0.02$

Figure 2. Disease-Free Survival, $p=0.04$

Figure 3.

Ambiguous Spitzoid Tumor. A- Low power view, 40× H&E. B- Deep nests of poorly maturing spitzoid melanocytes, 200× H&E. C- Scattered mitotic figures (arrowhead) were present in the lower half of the neoplasm, 400× H&E.

Table 1
Case Characteristics

	Study cohort (n=144)	FISH negative cases (n=26)	FISH positive cases (n=118)	p-value
Age at biopsy in years (range)	61 (17-97)	54 (17-82)	62 (28-97)	0.049
Male/female	56/44	58/42	55/45	0.831
Tumor thickness in mm (range)	3.7 (2-14)	4.3(2-12)	3.8 (2-14)	0.883
Cases with ulceration	55 (38%)	7 (27%)	48 (41%)	0.254
SLN status				1.0
Positive	35 (24%)	5 (19%)	30 (25%)	
Negative	81 (56%)	14 (54%)	67 (57%)	
NA *	28 (19%)	7 (27%)	21 (18%)	
Anatomic site				0.971
Head & Neck	45 (31%)	9 (35%)	36 (31%)	
Trunk	37 (26%)	7 (27%)	30 (25%)	
Extremities	62 (43%)	10 (38%)	52 (44%)	
Mean follow up in months (range)	32 (0-120)	35.7 (0-95)	31.3 (0-120)	

* NA-not available or patient declined sentinel lymph node biopsy

Table 2

Melanoma Subtypes.

Melanoma Subtype		Melanoma Subtype	
Superficial spreading	51	Chronic sun damage	43
Lentigo maligna	26	Non-chronic sun damage	82
Nodular	27	Acral	14
Acral lentiginous	8	Not classifiable	5
Not classifiable-Spitzoid features	326		

Table 3
Optimal Chromosomal aberration thresholds for current study cohort

	6p25 gain	6p25>Cep6	Myb<Cep6	11q13 gain
Established criteria ⁹	>29% cells	>55% cells	>40% cells	>38% cells
Criteria to best predict metastatic potential in this study	>28% cells	>45% cells	>55% cells	>11% cells