Summary. The large majority of melanocytic lesions can be reliably classified as either benign or malignant based upon morphology alone, but a minority of lesions remains difficult to classify by traditional histologic methods. Recently, a panel of fluorescence in situ hybridization (FISH) probes targeting loci on chromosomes 6 and 11 has emerged as a powerful tool to discriminate melanoma from nevi. This has been validated in numerous difficult diagnostic scenarios. In addition, this same FISH panel has been shown to provide independent prognostic information in traditional melanomas. There is accumulating evidence that FISH targeting these loci as well as several other key chromosomal loci such as 9p21 and 8q24 can provide valuable prognostic information in histologically ambiguous melanocytic tumors. However, since the vast majority of atypical spitz tumors have an indolent course, larger studies including adequate numbers of cases with adverse events is necessary to provide sufficient proof of its role in clinically relevant cases. In this review, we discuss the current literature and studies to date on this topic.

Key words: Fluorescence in situ hybridization, Melanoma, Spitz, Nevus

Introduction

The large majority of melanocytic lesions can be reliably classified as either benign or malignant based upon morphology alone. However, a minority of melanocytic lesions remains difficult or impossible to classify by standard histopathology. There are likely at least 2 reasons for this diagnostic dilemma one of which includes the existence of a poorly characterized subgroup of melanocytic neoplasms of low or intermediate grade malignant potential. Melanocytic neoplasms such as pigmented epithelioid melanocytoma and some atypical spitz tumors likely fit in this category. The second source of diagnostic challenge is based on the fact that some highly malignant neoplasms with high likelihood of aggressive clinical behavior such as some nevoid melanomas may have conflicting histopathologic features making it difficult to render a definitive diagnosis and some totally benign nevi may have some morphologic features raising concern for melanoma. The dermatopathology literature is replete with examples of studies profiling these issues and of the lack of concordance between expert dermatopathologists in the diagnosis of these subsets of melanocytic neoplasms (Barnhill et al., 1999, 2008; Gerami et al., 2010a). Incorrect diagnosis of a melanocytic neoplasm has dramatic implications for morbidity and mortality. Under diagnosis of a malignant neoplasm may result in withholding potentially life saving therapy, while over diagnosis exposes the patient to morbidity associated with unnecessary invasive procedures and being labeled as having a potentially lethal cancer.

Based on data from comparative genomic hybridization, for over ten years, we have known that melanomas carry frequent chromosomal copy number aberrations, including copy number gains at 1q, 6q, 7, 8q, 17q, and 20q as well as deletions at 6q, 8p, 9p, and 10q (Bastian et al., 1998, 2003). Benign nevi, on the other hand, typically lack chromosomal copy number aberrations and are diploid with the exception of small subsets of Spitz nevi, which may have specific recurring copy number aberrations. More specifically, approximately 20% of spitz nevi may have copy number gains at 11p where the HRAS gene is located (Bastian et al., 2000). These spitz nevi are typically large bulky lesions with prominent sclerosis of the deep dermal component. Alternatively another group of spitzoid lesions consisting of a an expansile proliferation of dermal epithelioid melanocytes with spitzoid cytomorphic features may harbor copy number
losses of 3p21 where the BAP1 gene is located (Wiesner et al., 2012). Hence there is considerable data showing that melanomas differ from nevi when evaluating chromosomal copy number aberrations. The identification of known characteristic targets has become a springboard for the development of targeted diagnostic FISH assays.

Recently fluorescence in situ hybridization (FISH) has emerged as a powerful tool for differentiating melanomas from benign melanocytic nevi. In 2009, a panel of 4 FISH probes targeting 3 loci on chromosomes 6 and one on chromosome 11 was introduced to dermatopathologists and has subsequently become CE marked in Europe. Validation studies have documented a sensitivity of anywhere between 82% and 94% with a specificity of anywhere 90 and 98% (Gerami et al., 2009a, 2010b; Morey et al., 2009; Moore and Gasparini, 2011). It has been subsequently shown that the sensitivity for spitzoid melanomas can be further optimized by including a probe targeting 9p21 with Cep9 or another reference probe, while probes targeting 8q24 would be of significant benefit for amelanotic nodular nevoid melanomas (Gammon et al., 2012a; Pouryazdanparast et al., 2012). Hence a newer probe set including 6p25, 11q13, 8q24 and 9p21 maybe commercially available soon. In addition to better targeting of spitzoid and some amelanotic nevoid melanomas, this set also is less likely to give false positives as a result of tetraploidy because of the targeting of loci on 4 different chromosomes. In validation studies, this set of probes performed with 94% sensitivity and specificity of 98% (AJSP in press, accepted Dec 2011).

The CE marked FISH assay targeting chromosomes 6 and 11 has further been validated in a number of specific diagnostically challenging scenarios. This includes distinguishing atypical cellular blue nevi from blue nevus-like melanomas, differentiating epitheloid blue nevi from blue nevus-like metastases, nevoid melanomas from mitotically active nevi, conjunctival nevi from conjunctival melanoma, and sclerosing nevi from desmoplastic melanomas (Gerami et al., 2009b, 2010c, 2011a; Pouryazdanparast et al., 2009; Busam et al., 2010; Gammon et al., 2011).

As FISH sections can be easily compared and correlated with histologic images, FISH has also been used in microstaging of melanomas arising in association with a nevus. Accurately measuring the Breslow depth of melanomas with a prominent nevoid dermal component is a common challenge to dermatopathologists. In a series of 36 melanomas with a significant nevoid dermal component, 28 (78%) tested positive by FISH in the histologically malignant areas, while the benign nevoid components were universally negative for chromosomal aberrations at the tested loci (Newman et al., 2009). These results suggest that FISH may be useful to provide accurate Breslow depth in this common clinical scenario, and thereby provide more accurate prognostic information to the clinician and patient.

Similarly because of the ability to identify chromosomal copy number aberrations in small aggregates of cells FISH has also shown promise in distinguishing nodal nevi from melanoma in sentinel lymph node biopsies. In a series of 41 patients with sentinel lymph node biopsies, 24 of which had metastatic melanoma and 17 of which had nodal nevi, FISH detected chromosomal aberrations in twenty of 24 (83%) of the metastases, and one of the 17 intranodal nevi (Dalton et al., 2010). Importantly, in the nodal nevus positive by FISH, multiple chromosomal aberrations were present that were also present in the primary melanoma, suggesting that the metastasis was deceptively histologically bland and misclassified as a nevus (Dalton et al., 2010).

Several studies have indicated that identification of copy number aberrations by FISH may also provide significant prognostic information in conventional melanomas. In a series of 144 melanomas of Breslow depth >2mm, FISH-positive lesions had a significantly increased risk of metastasis or melanoma-specific mortality when compared to the FISH-negative lesions (North et al., 2011). A separate study showed that among a series of potential chromosomal copy number aberrations seen in cutaneous melanoma, that copy number gains of CCND1 and MYC at loci 11q13 and 8q24 were most prognostic. In a case-control study of 55 metastasizing and 42 non-metastasizing melanomas of similar Breslow depths, gains at 8q24 and 11q13 were second only to ulceration in their prognostic potential (Gerami et al., 2011b). Amplification of CCND1 maybe seen in the form of homogeneous staining regions (HSR) and when present seem to consistently correlate with an adverse prognosis. We previously reported the case of a relatively thin melanoma with Cyclin D1 HSR that followed an aggressive course (Gerami et al., 2008). We subsequently reported similar results in a follow up series of 7 additional patients (Gammon et al., 2012b).

The ultimate goal of any adjunctive diagnostic molecular test for melanocytic neoplasms is to better predict clinical behavior in lesions with ambiguous histopathologic features. There is considerable accumulating evidence that tools such as CGH and FISH which can identify chromosomal copy number aberrations are predictive of clinical behavior however there has been some variability in this assessment by different investigators. This is particularly true for atypical spitz tumors. There are a number of reasons underlying this variability. The first and foremost of these is our current incomplete and continuously evolving understanding of atypical spitz tumors.

For many years it was believed that the presence of melanocytes in the lymph node from patients with atypical spitz tumors was evidence that the lesion was in fact a melanoma. However, a series of 67 patients with atypical Spitz tumors, of whom 57 underwent sentinel lymph node biopsy (SLNB), revealed that 27 (47%) of patients had involvement by the sentinel nodes with atypical spitz tumor (Ludgate et al., 2009). Despite this, all 27 patients with positive SLNB were alive with no
evidence of disease at last follow up (Ludgate et al., 2009). Subsequently, other studies have confirmed that SLNB is commonly positive in AST, but does not carry the same prognostic significance as it does conventional melanomas (Busam et al., 2009; Ghazi et al., 2010). It is in part because of these studies that it has become more accepted that some atypical spitz tumors are likely an intermediate grade tumor with frequent involvement of the sentinel nodes but only infrequent distant metastasis or death of the patient. In fact the incidence of distant metastasis or death of disease for such cases is quite rare. As even collaborative studies involving multiple major tertiary care centers often struggle to identify a handful of such cases as the vast majority of atypical spitz tumors do not result in distant metastasis or death.

In a series of 27 patients with atypical spitz tumors, we identified 6 cases with either bulky lymph node metastasis, distant metastasis or death. In 2 of the 6 cases, the disease was limited to lymph node metastasis alone. Hence our study included only 4 patients with distant metastasis or death. FISH studies using the conventional CE marked probe set for melanoma targeting 6p25, 6q23, Cep6 and 11q13 was positive in 6 of 6 cases as well as 6 of the 21 non-metastasizing atypical spitz tumors. Using a Fisher’s exact test comparing the frequency of metastasis in the FISH positive versus the FISH negative cases resulted in a highly significant p value of 0.003. In a European study of 113 ambiguous melanocytic lesions led by Vergier et al., 13 patients had either distant metastasis or death of disease. They also concluded that FISH in conjunction with histologic evaluation improved diagnostic accuracy. Massi et al reported 38 atypical spitz tumors with only one case having distant metastasis and death. This one case did show multiple chromosomal copy number aberrations detectable by FISH.

Studies by Gaiser et al. and Raskin et al. had different findings. Gaiser, et al. studied 12 histologically ambiguous lesions classified as benign or malignant based upon clinical endpoints alone. Gaiser, et al. concluded that FISH did not reach clinically useful sensitivity or specificity (Gaiser et al., 2010). Interestingly, the investigators had some discordant CGH and FISH data. Copy number changes from a specific locus were identified by CGH but not by FISH though the locus was targeted by the FISH assay. Since generally at least 30-50% of cells collected need to have a specific chromosomal aberration for it to be detectable by CGH, it should certainly also be detectable by FISH which can identify much smaller aberrant tumor populations (Bauer and Bastian, 2006). This raises concern for methodologic difficulties. Furthermore, it should be noted that the investigators did not use the same criteria reported in the multi-site FISH validation study. Finally, specificity for metastatic behavior is an unfair measure since even the gold standard histology would have a low specificity for metastatic behavior. Even frankly malignant melanomas of intermediate stage (T2 or T3) identified by standard histology will only metastasize in 40 to 60% of cases while the others are cured by surgery alone. The series from Raskin, et al, included 16 patients with AST, of whom 15 were alive with NED at last follow up, while 1 died of widely metastatic disease. All 16 were negative for copy number aberrations at chromosomes 6 and 11 (Raskin et al., 2011). The FISH studies in this case were performed by an outside laboratory and not by the investigators themselves.

In conclusion, among these studies, the cross European study clearly had the highest number of cases with adverse events beyond the lymph node which was 13, while the remaining studies had significantly fewer patients. This again underscores the infrequency with which these intermediate grade tumors result in distant metastasis and death. This is in part the difficulty with trying to design a diagnostic test targeting these intermediate grade tumors. We are hoping to find a molecular signature that will identify that rare case among many intermediate grade tumors that will end up in a catastrophic outcome. This maybe an unrealistic task since even among frankly malignant melanomas of stage T2 or T3, only approximately 40% to 60% may result in metastasis while the remaining cases are cured by surgery alone. Hence in dealing with a tumor of lower grade malignant potential with only rare distant metastasis a significantly greater proportion would be expected to be cured by surgery alone. A more realistic expectation is to identify subgroups of atypical spitz tumors with specific copy number aberrations that are much more likely or at relatively greater risk to result in distant metastasis and death. Most of the above studies suggest that FISH positive spitz tumors have a higher likelihood of aggressive clinical behavior than FISH negative spitz tumors. A test that provides this information alone is a significant step forward in helping us better classify and predict prognosis in atypical spitz tumors. Additionally, there is no question that FISH would be helpful in identifying cases of frank melanoma mis-classified as an atypical spitz tumor on initial histologic inspection. Further studies including a broader range of probe targets and more atypical spitz tumors resulting in distant metastasis or death would be helpful in further establishing this.

References


detected by comparative genomic hybridization. Cancer Res. 58, 2170-2175.


Accepted August 27, 2012