Development and Validation of a Fluorescence In-situ Hybridization (FISH) Based Assay as an Adjunct to Histopathology in the Diagnosis of Melanoma

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ABSTRACT

Melanoma lesions exhibit significant morphological heterogeneity. While the majority of biopsies can be classified as benign (nevus) or malignant (melanoma) using well-established histopathological criteria, there exists a cohort for which the prediction of clinical behavior and invasiveness or metastatic potential is difficult or impossible on the basis of morphological features alone. Studies have shown that there is significant disagreement between pathologists, and even expert dermatopathologists, in the diagnosis of this subgroup of difficult melanocytic lesions. Recent studies have identified several genomic and chromosomal aberrations constant within melanoma, but absent from melanocytic nevi. Presented will be an outline of the steps taken to clinically validate a three locus, 4-color FISH based assay for the differentiation of melanoma from benign melanocytic nevi. Included within the presentation will be a review of the validation cohort, FISH automation strategy, signal interpretation and scoring criteria.

INTRODUCTION

Melanoma is a serious and dangerous form of skin cancer. The accuracy of its diagnosis continues to challenge dermatopathologists even with modern-day histopathological techniques. Much of the discordance in the diagnosis center around the dysplastic nevus which harbor only subtle discriminating phenotypic characteristics (i.e. non-melanomas). Recent studies have found that there are more unique differences that exist on the genetic level and have posed evidence that a diagnostic FISH test specific to this has some clinical utility[1,2,3,4,5]. This study is the investigation and validation of a four-probe FISH assay (MelanoSITE™) detecting signal alterations with Abbot/Vysis probes USMB (6p25; SpectroFluor, USMB) and CEP 6 (5µm tissue sections were baked at 65°C for 20 min and deparaffinized through multiple xylenewashes and dehydrated with ethanol. Specimen slides were air dried and subsequently passed through a series of pretreatment washes on the VP2000 Processor (Abbott Molecular). 1X SSC (30°C, 35 min), DI water (3 min), protease solution (10% Pepsin in 0.2N HCL; 37°C for 34 min), DI water (3 min), increasing ethanol series (70%, 80%, 100% ethanol, 2 min each). The probe cocktail, containing the fluorescently labeled probes for sequence specific hybridization, was dispensed onto the specimen and covered with a cover gass. Automated densitometry was performed on the Thermostar (Abbott Molecular) at 60°C for 8 min followed by the hybridization at 37°C for 16-18 hrs. The cover gass was carefully removed from the tissue specimen. The post-hybridization wash procedure on the VP2000 consisted of 2X SSC with 1% Tween (70°C, 2 min) and 0.5% SSC. Specimen slides were allowed to sit until solution until the AFI I counterimmunoperoxidase.

RESULTS

Molecular): 1X SSC (80°C, 35 min), DI water (3 min), protease solution (10% Pepsin in 0.2N HCL; 37°C for 34 min), DI water (3 min), increasing ethanol series (70%, 80%, 100% ethanol, 2 min each). The probe cocktail, containing the fluorescently labeled probes for sequence specific hybridization, was dispensed onto the specimen and covered with a cover glass. Automated densitometry was performed on the Thermostar (Abbott Molecular) at 60°C for 8 min followed by the hybridization at 37°C for 16-18 hrs. The cover glass was carefully removed from the tissue specimen. The post-hybridization wash procedure on the VP2000 consisted of 2X SSC with 1% Tween (70°C, 2 min) and 0.5% SSC. Specimen slides were allowed to sit until solution until the AFI I counterimmunoperoxidase.

DISCUSSION

The MelanoSITE™ assay was determined to be 97% and 77%, respectively. This was derived from the results from the Nevi and Melanoma groups. Specificity values for the scale nev (92%) and the spitz nevi (90%) subgroups were slightly lower. Reproducibility tests were reported at 100%. The MelanoSITE™ assay was found to be highly reproducible with duplicate cases yielding the same results in 2 independent hybridization runs.

REFERENCES