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Letters to the Editor

A cautionary note on the immunohistochemical detection of braf v600e mutations in serrated lesions of the colon

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To the editor: We read with great interest the recently published article by Mesteri *et al*[1] regarding immunohistochemical detection of *BRAF* mutations in serrated colonic polyps. Some types of serrated polyps, particularly sessile serrated polyps (sessile serrated adenomas), frequently harbor *BRAF* c.1799T>A (p.V600E) mutations and presumably represent precursor lesions to sporadic colonic carcinomas with a high degree of microsatellite instability (MSI-H). Detection of *BRAF* mutations helps distinguish sporadic MSI-H tumors from those associated with Lynch syndrome since Lynch-related adenocarcinomas virtually never harbor *BRAF* mutations. *BRAF* status combined with testing for microsatellite instability also provides important prognostic information. Recent evidence indicates that MSI-H tumors with wild-type *BRAF* have a better prognosis than those associated with *BRAF* mutations. Patients with microsatellite stable (MSS) tumors and mutations in *BRAF* have lower 5-year survival than those with MSI-H tumors and those with *BRAF* wild-type MSS tumors.[2] (For a complete discussion of the molecular carcinogenesis in colorectal carcinoma, the reader is referred to a recent review by Colussi *et al*.[3]) Evaluation of *BRAF* is currently limited to molecular techniques, such as PCR and sequencing assays, which often require specialized laboratories and are not widely available to practicing pathologists. Thus, simple and inexpensive methods to evaluate *BRAF* mutational status are of substantial clinical interest. The potential clinical utility of detecting *BRAF* mutations in sessile serrated polyps is less clear. Although up to 80% of sessile serrated polyps and microvesicular hyperplastic polyps contain mutated *BRAF*, most of these do not progress to adenocarcinoma; thus, *BRAF* mutations are neither diagnostic of sessile serrated polyps, nor do they reliably identify polyps that are at risk for malignant progression.[4,5]

In this study, Mesteri *et al* used the *BRAF* p.V600E mutation-specific antibody, VE1, to subclassify serrated polyps according to *BRAF* mutational status. This antibody was originally developed in the laboratory of one of the co-authors, Dr Andreas von Deimling, and is now commercially available. The authors correlated their immunohistochemical findings with the results of sequencing assays in a subset of these polyps. They reported positive VE1 staining in 100% of sessile serrated polyps and the majority of traditional serrated adenomas and microvesicular hyperplastic polyps, as well as perfect correlation between immunohistochemical and sequencing assays. The authors concluded that immunohistochemistry is a feasible means of detecting *BRAF* mutations in serrated polyps in routine practice. The immunostaining results depicted by Mesteri *et al* are convincing and strongly support their claim. However, we would like to compare their experience with the VE1 antibody with that of our group and point out major obstacles to the routine use of this antibody that were not addressed by these authors.

We recently evaluated *BRAF* mutational status by sequencing and immunohistochemistry in sessile serrated polyps, using the commercially available VE1 antibody from Spring Bioscience (Pleasanton, CA, USA). We first tested seven sessile serrated polyps with *BRAF* c.1799T>A (p.V600E) mutations, including two with cytologic dysplasia, as well as one sessile serrated polyp that was *BRAF* wild-type by sequencing. Cytoplasmic staining was observed in only one of seven mutation-positive cases when using the VE1 antibody at 1:50 dilution, as recommended by the manufacturer, and the staining reaction was focal and weak. All eight cases also showed moderately intense nuclear staining in both lesional and non-lesional colonic epithelial cells, thereby complicating interpretation. We then carried out additional experiments using various antigen retrieval methods and concentrations in order to optimize the antibody, but these measures failed to improve stain sensitivity. Overall, we found that VE1 immunostaining was concordant with molecular analyses in only 3 (7%) of 43 *BRAF*-mutated serrated polyps.[6] Thus, in our hands, the results reported by Mesteri *et al* are not reproducible when the commercially available VE1 antibody is used.

Previous studies evaluating this antibody in invasive colonic adenocarcinomas have also produced mixed results. Affolter *et al*[7] reported 100% concordance between immunohistochemical VE1 stains and pyrosequencing of *BRAF* V600E in colorectal adenocarcinomas, but noted that staining was either weak or heterogeneous in 43% of *BRAF*-mutated tumors. Sinicrope *et al*[8] also reported complete concordance between VE1

staining and *BRAF* mutational status, although weak staining of 10–30% of tumor cells was considered a 'positive' result in 22% of *BRAF*-mutated cancers. Other authors have observed false-positive and false-negative results with the VE1 antibody. Adackapara *et al*[9] reported weak staining in 26% of *BRAF* wild-type colorectal carcinomas, whereas 29% of *BRAF*-mutated tumors were negative and 35% showed weak staining. Finally, Kuan *et al*[10] observed VE1 staining in 54 of 57 (95%) invasive adenocarcinomas with *BRAF* mutations, but also found that three of four tumors with weak cytoplasmic staining proved to have wild-type *BRAF* by molecular analysis.

The critical difference between our immunohistochemical staining methods and those of Mesteri *et al* reflects the nature of the antibody utilized. Mesteri *et al* used undiluted VE1 hybridoma supernatant for their experiments, which is available only to Dr Andreas von Deimling and collaborators. In contrast, the commercially available VE1 antibody (Spring Bioscience) that we and others have used was produced by protein A/G purification of the VE1 hybridoma antibody and reconstituted in Tris-HCl pH 7.5 with carrier proteins. The product specification sheet does not indicate whether the antibody used for purification was obtained through hybridoma supernatants or in ascites form, and the antibody concentration of this mouse IgG2a antibody is also not specified.

We suspect that the difference between the original research antibody and the commercial antibody from Spring Biosciences is the fundamental reason why our results are so different from those of Mesteri *et al*. We conclude that routine use of the currently available VE1 antibody for detection of BRAF p.V600E in serrated colonic polyps and invasive colonic adenocarcinomas is premature for several reasons. First, most serrated polyps that harbor *BRAF* mutations do not show VE1 immunohistochemical expression. Second, others have reported that occasional invasive colonic adenocarcinomas with mutated *BRAF* are negative for VE1, whereas weak or focal VE1 staining occurs in both *BRAF* wild-type and *BRAF*-mutated tumors. Last, but not least, the VE1 antibody is a costly immunohistochemical stain ($2000 per 0.5 ml), which mitigates its potential advantage over molecular analyses. We are aware that Ventana Medical Systems (Tucson, AZ, USA) now markets a VE1 antibody as well. It may be worthwhile to evaluate the performance of this new antibody in *BRAF*-mutated carcinomas, including colonic adenocarcinomas, and in serrated colonic polyps.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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References

1 Mesteri I, Bayer G, Meyer J, *et al.* Improved molecular classification of serrated lesions of the colon by immunohistochemical detection of BRAF V600E. Mod Pathol 2014;27:135–144.
2 Lochhead P, Kuchiba A, Imamura Y, *et al.* Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. J Natl Cancer Inst 2013;105:1151–1156.
3 Colussi D, Brandi G, Bazzoli F, *et al.* Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. Int J Mol Sci 2013;14:16365–16385.
4 Spring KJ, Zhao ZZ, Karamatic R, *et al.* High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. Gastroenterology 2006;131:1400–1407.
5 O'Brien MJ, Yang S, Mack C, *et al.* Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. Am J Surg Pathol 2006;30:1491–1501.
6 Weidner A-S, Panarelli N, Yantiss R, *et al.* Immunohistochemical and molecular evaluation of *BRAF* mutations in tumors of the serrated neoplastic pathway. Mod Pathol 2014;27:209A.
7 Affolter K, Samowitz W, Tripp S, *et al.* BRAF V600E mutation detection by immunohistochemistry in colorectal carcinoma. Genes Chromosomes Cancer 2013;52:748–752.
8 Sinicrope FA, Smyrk TC, Tougeron D, *et al.* Mutation-specific antibody detects mutant BRAFV600E protein expression in human colon carcinomas. Cancer 2013;119:2765–2770.
9 Adackapara CA, Sholl LM, Barletta JA, *et al.* Immunohistochemistry using the BRAF V600E mutation-specific monoclonal antibody VE1 is not a useful surrogate for genotyping in colorectal adenocarcinoma. Histopathology 2013;63:187–193.
10 Kuan SF, Navina S, Cressman KL, *et al.* Immunohistochemical detection of BRAF V600E mutant protein using the VE1 antibody in colorectal carcinoma is highly concordant with molecular testing but requires rigorous antibody optimization. Hum Pathol 2014;45:464–472.