**VE1 Antibody Immunoreactivity in Normal Anterior Pituitary and Adrenal Cortex Without Detectable BRAF V600E Mutations**

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**ABSTRACT**

**Objectives:** The VE1 monoclonal antibody was developed to recognize the V600E mutation in BRAF, which is found in various tumors.

**Methods:** We report that the VE1 antibody stains normal anterior pituitary gland and adrenal cortex, which lack detectable BRAF V600E mutations.

**Results:** Staining with the VE1 antibody was seen in the adenohypophysis and correlated well with adrenocorticotropic hormone (ACTH)–positive cells. ACTH-positive cells were typically most concentrated in the central mucoid wedge and pars intermedia, and VE1 staining was strong in these regions. Moreover, VE1 staining was seen in ACTH-expressing pituitary adenomas without detectable BRAF mutations. VE1 staining of the adrenal cortex was also significant, with the strongest staining seen in the inner segment of the zona fasciculata. Parathyroid glands, pancreatic islets, or parafollicular C cells in the thyroid showed no VE1 staining.

**Conclusions:** Overall, VE1 staining of endocrine tissues strongly suggests limitations on the use of this antibody for the detection of BRAF mutations.
Materials and Methods

Archival, formalin-fixed, paraffin-embedded materials on five samples each from postmortem pituitary glands, adrenocorticotropic hormone (ACTH)–producing pituitary adenomas, adrenal glands, parathyroid glands, pancreas, and thyroid glands were retrieved from the pathology files of Massachusetts General Hospital, Boston, MA.

Immunohistochemistry

Immunohistochemical studies were performed on 5-μm-thick sections of formalin-fixed, paraffin-embedded tissue in a Bond 3 automated immunostainer (Leica Microsystems, Bannockburn, IL), and primary antibodies against BRAF V600E (clone VE1, 1:100, Spring Bioscience, Pleasanton, CA), ACTH (AH26, 1:200, BioGenex, San Ramon, CA), prolactin (A0569, 1:1000, Dako, Carpinteria, CA), growth hormone (1:70, BioGenex), thyroid-stimulating hormone (5404, 1:5000, BioGenex), luteinizing hormone (3LH5B6YH4, 1:9000, BioGenex), follicle-stimulating hormone (83/12/2A82C7, 1:800, BioGenex), and human chorionic gonadotropin α subunit (823, 1:6500, BioGenex). The sections were deparaffinized on the Leica Bonds using Bond dewax solution. Leica Polymer Refine Kit was used for diaminobenzidine staining. Appropriate positive and negative controls were included.

Mutational Analysis

The SNaPshot genotyping assay for BRAF V600E and BRAF variant mutations developed by our group was performed on these tissues and consists of multiplexed polymerase chain reaction followed by a single-base extension reaction and uses the commercially available SNaPshot platform (Applied Biosystems, Carlsbad, CA).

Results

Immunohistochemistry

Staining with the BRAF V600E VE1 antibody was observed in all pituitary gland samples studied. Staining with the VE1 antibody was seen in the anterior lobe of the pituitary gland but not in the posterior lobe Image 1A. Notably, strong VE1 staining of a subset of anterior pituitary cells was seen in a background of variably weak staining in most of the other cells Image 1B and Image 1C. To determine which hormone-producing cells strongly reacted with VE1 antibody, a standard pituitary hormonal panel (ACTH, prolactin, growth hormone, thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, and α subunit) was performed on the postmortem pituitary glands. The intensity and distribution of the VE1 staining correlated well with ACTH-positive cells Image 1D, Image 1E, and Image 1F in the anterior lobe. Moreover, ACTH-positive cells are typically most concentrated in the central mucoid wedge and pars intermedia, and VE1 staining was strong in these regions. So-called “basophil infiltration”—in which anterior pituitary cells, primarily ACTH-producing cells, “invade” the posterior lobe—is a common age-related finding. In this regard, it is significant that the scattered ACTH-positive cells in the posterior lobe were also strongly stained with the VE1 antibody (Image 1). This pattern of cross-reactivity of ACTH-producing cells with the VE1 antibody was observed in all pituitary glands studied and in the ACTH-producing pituitary adenomas.

Given that VE1 immunoreactivity was present in the endocrine portion of the pituitary gland, additional endocrine organs were tested with this antibody. Parathyroid glands, pancreatic islets, and parafollicular C cells in the thyroid showed no VE1 staining. On the other hand, VE1 staining of the adrenal cortex, but not of the adrenal medulla, was significant Image 2. The strongest VE1 staining was seen in the inner segment of the zona fasciculata and to a lesser degree in the zona reticularis, which borders the medulla.

Mutational Analysis

Mutational analyses for BRAF V600E, V600M, V600K, L597S, L597V, G466V, and G469V mutations were negative in five pituitary gland, ACTH-producing pituitary adenoma, and adrenal gland samples.

Discussion

Mutant-specific antibodies are a powerful tool to gain important diagnostic and prognostic information on specimens, especially when tissue specimens available for genetic studies are limited. The BRAF V600E antibody VE1 has been demonstrated to recognize this common oncogenic mutation in various tumor types. We now report that this antibody also recognizes some normal endocrine tissues. Staining of the anterior, but not posterior, pituitary gland was consistent, with the strongest staining in ACTH-positive cells. This raises the possibilities that the antibody recognizes an epitope that is highly expressed in ACTH-producing cells, and to a lesser degree in other pituitary cells, or also recognizes an additional epitope in ACTH-negative cells. In addition, VE1 immunoreactivity was observed in the adrenal cortex, especially in the zona fasciculata, which contains cortisol-producing, ACTH-responsive cells. This suggests that this antibody may be recognizing an epitope common to cells of the ACTH-cortisol hormonal axis.

We observed strong staining of VE1 in the ACTH-producing adenomas associated with Cushing disease. Genotyping for
**Image 1** Corresponding cells in anterior pituitary stained with VE1 (BRAF V600E) (A, B, and C) and adrenocorticotropic hormone (D, E, and F) immunostains in three postmortem specimens of pituitary glands.
To identify proteins that might contain significant homology with mutant \(BRAF\) V600E, protein searches were performed using the Basic Local Alignment Search Tool, which did not reveal any endocrine-related candidate protein. For example, human AlkB homolog 7 shares an identical seven-amino acid region within the 11-amino acid immunogen used to produce the VE1 antibody, but it is a widely expressed nuclear-encoded mitochondrial protein.

One possibility is that VE1 antibody may also recognize other mutant forms of \(BRAF\). In our previous study, one metastatic melanoma in the brain with V600K (p.Val600Lys) mutation (confirmed with repeated mutational analyses) was positive for monoclonal VE1.\(^{13}\) This could be because of a protein conformation change that is close enough to be picked up by the antibody. However, genotyping for all \(BRAF\) variant mutations (including V600K) was negative in this study for all cases studied.

Overall, these findings urge caution when using the VE1 antibody for detecting primary or metastatic tumors in the pituitary and adrenal glands. The use of this antibody as a screening tool for \(BRAF\) V600E mutation in tumors in the absence of concordant genetic testing may risk false-positive results.

**References**


