

Immunohistochemical Detection of BRAF in Iraqi Women with Different Breast Tumors

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Abstract: ***Background:** Breast cancer is the most common form of neoplasm and is leading cause of cancer death among women worldwide. This study examined the impact of BRAF in Iraqi breast cancer patients and explored the association between the BRAF expression and clinicopathological parameters. **Aim:** The aim of this study was to determine the BRAF status and investigate its relation to histopathological parameters. **Patients and Methods:** A total 60 retrospective breast cases were studied, 47 out of 60 were malignant tumors, 7 out of 60 cases were benign tumors and 6 samples of normal breast tissue as control. The malignant cases were graded according to the modified Bloom and Richardson criteria into three histological grades. We used immunohistochemistry to evaluate the expression BRAF in relation to histopathological factors. **Statistical Analysis:** Statistical analysis was done by using T-test and Chi-square test to calculate p-value to ascertain statistical significance. **Results:** In 47 breast cancer sections, 31 (65.96%) of cases showed positivity for BRAF specific antibody, while only 2(28.57%) of 7 benign cases showed positive expression when stained with this marker in comparison with 6(100%)normal breast tissue samples were showed totally negative expression of BRAF Results reported high significant correlation between BRAF expression with(age and tumor stages)but insignificant with tumor grades. **Conclusion:** These results revealed the presence of BRAF mutation in a proportion of breast cancer, and BRAF mutations showed association with some clinicopathological factors.*

Keywords: Breast cancer, BRAF, Immunohistochemistry

1. Introduction

Breast cancer is the most common cause of cancer-related deaths in women (1). It starts when cells in the breast begin to grow out of control. This cancer usually starts in the inner lining of milk ducts or the lobules that supply them with milk (2). A malignant tumor can spread to other parts of the body, while benign breast tumors are abnormal growths, but they do not spread outside of the breast and they are not life threatening. But some benign breast lumps can increase a woman's risk of getting breast cancer (3).

The predominant cause of death in patients is the ability of cancer cells to invade surrounding tissues and form distant metastases (4). Breast cancer is well known to have genetic aberration and genetic heterogeneity (5). BRAF is a member of Raf kinase family proteins, and it is the most important activator of MEK kinase in Ras-Raf-MEK-ERK pathway (6). In certain tumors, this pathway is abnormally activated; the BRAF mutation is a typical cause of aberrant ERK signaling (7). BRAF mutation was first recorded in 2002 and has been reported to be found in various neoplasms, and the reported prevalence in tumors was as follows; malignant melanoma (40-70%), colorectal carcinoma (5-22%), thyroid papillary carcinoma (36-53%) and lung adenocarcinoma (4%) (8). In previous studies, about 10% of breast cancer cells had BRAF mutation (9), suggesting the possible presence of BRAF mutation in breast cancer tissues. The common method for detecting BRAF mutation is the Sanger sequencing method, but the test is expensive and it requires expensive equipments. To

overcome these problems, immunohistochemistry (IHC) method using BRAF specific antibody was introduced (10). A few studies on BRAF in breast cancer tissues have been performed so far, using sequencing methods but studies that used BRAF specific antibody are rare (11). This study was designed to investigate the expression of BRAF status in different breast tissues by using immunohistochemistry and its' clinical implications.

2. Patients & Methods

Specimens' collection

A total of 60 cases of breast biopsies embedded in paraffin blocks were collected from the archive of Histopathology Unit of Al-Yarmouk Teaching Hospital in Iraqi capital Baghdad. Among these, 47 cases of malignant tumors, 7 cases of benign lesions, and 6 cases reveal no significant pathology. Clinical information about patient's age, tumor size, grade, and pathological stage was obtained from the available histopathological reports. Hematoxylin and Eosin (H&E) stained sections were re-examined by a pathologist. Each block was cut at a thickness of 5µm on a microtome cutter (Leica RM2235). Sections were then placed on salinized coated slides, (DAKO, UK) and heated at 58°C for 24 hours.

Immunohistochemistry

IHC was carried out according to standard protocols. Briefly, sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol (100%, 90%, 80%, and 70%). Then, Antigen was retrieved using

Antigen retrieval solution (15 ml of ready to use solution diluted with 150 ml of distilled water) and slides were put in this solution inside pressure cooker and heated in scientific microwave for 10 min. Next, sections were encircled with wax pencil around the tissues. And slides were incubated in peroxidase -blocking solution (Dako, ready- to- use) for 20 minutes. Rabbit monoclonal antibodies kit (ab33899, ABCAM, UK) was used to detect primary antibody of BRAF, which were diluted (1:250) using antibody diluent (ready-to-use, Code No.ab64211 Abcam, Cambridge, UK), and incubated for 1hr. at room temperature according to kit producer. After that, Secondary antibodies for BRAF (Anti Rat Ig. peroxidase, Cat. No. MP-7404, ImmPRESS™ Vector, USA) were applied to the slides and incubated for 30 minutes at room temperature in a humidified chamber. The colorimetric detection of reaction was achieved by the Diaminobenzidine (DAB) Peroxidase Substrate method. Then, sections were counterstained with haematoxylin, dehydrated, and mounted. Finally, the slides were scanned and scored by a pathologist.

Interpretation of Immunohistochemical staining

The positive expression for BRAF was observed as a diffuse brown cytoplasmic staining as well as nuclear staining. Sections with 20% or more positive tumor cells were recorded as BRAF positive according to (11, 12).

Statistical analysis

Statistical analysis was carried out by using (SPSS V. 20). T-test and Chi square were used to assess the association among clinical-pathological features and the expression of BRAF tissue. *P* values ≤ 0.05 were considered to be statistically significant.

3. Results

Patient's age ranged from (25-50) years, with a mean of (50.72±11.82) years in malignant group, while the mean age in benign group was (25.57±7.18). The peak age rate in malignant group was in the age group (41-50) years (Figure 1).

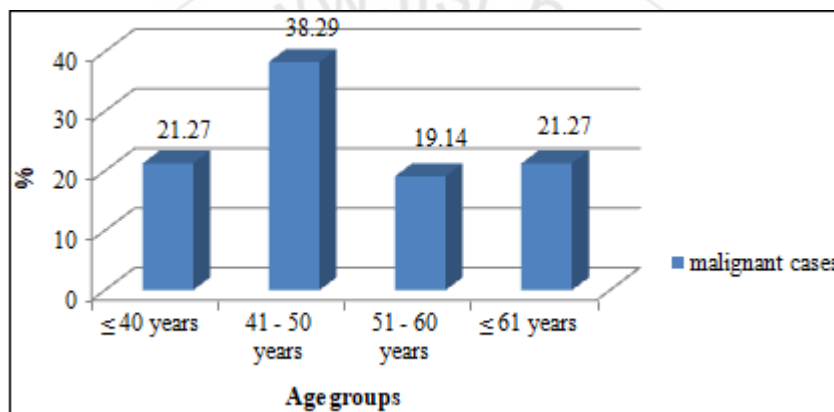


Figure 1: Distribution of cases according to age groups

a) Immunohistochemical expression of BRAF patients groups

In current study, BRAF expression was positive in 2(28.57%) of benign sections and 31(65.96%) of malignant sections while it was totally negative expression in normal

breast tissue sections (figure2). Twenty two (71%) of malignant sections showed cytoplasmic expression while 9(29%) showed nuclear expression (figure 3).

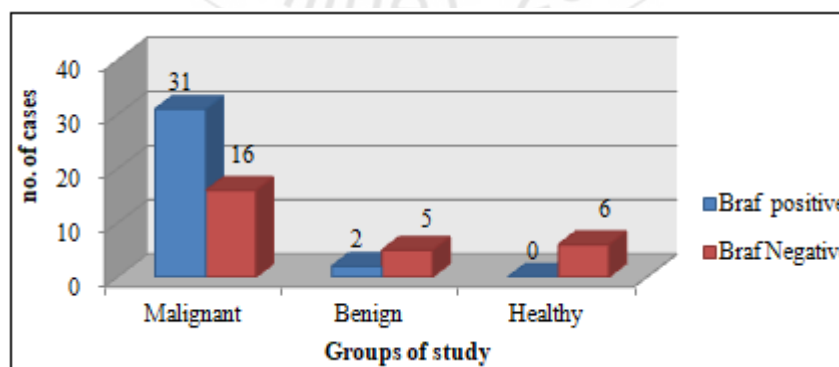


Figure 2: IHC Expression of BRAF in patients groups

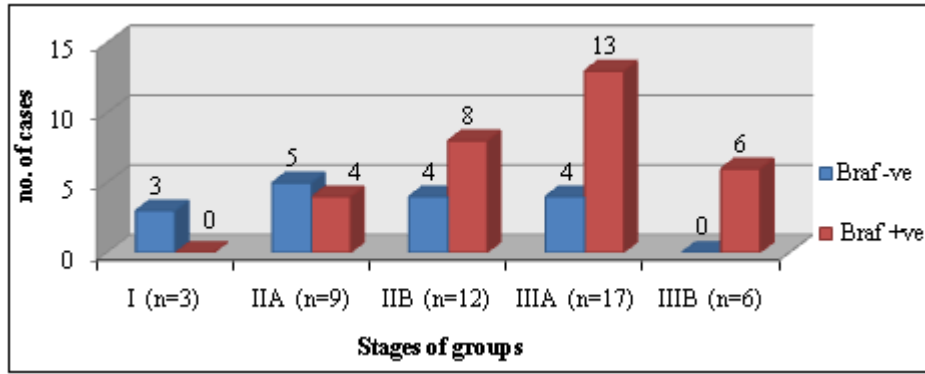


Figure 3: IHC expression of BRAF in malignant cases

b) Immunohistochemical expression of BRAF according to age group:

Results showed high significant correlation ($P=0.00000126$) between BRAF expression and the age in malignant cases,

the largest number of cases was at age group (41-50) years (Figure 4).

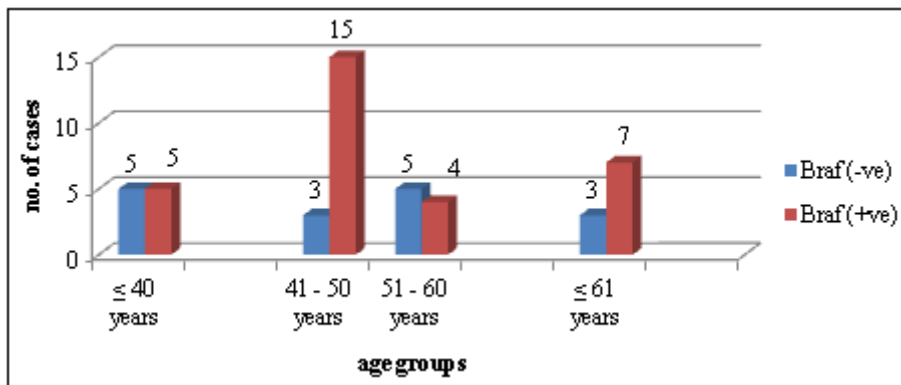


Figure 4: IHC Expression of BRAF in malignant group according to age

c) Immunohistochemical expression of BRAF according to tumor stage:

Results showed high significant correlation ($P=0.000534$) between expression of BRAF with different tumor stages. That is, all cases in stage I of disease showed negative

expression in comparison with late stage of disease (stage IIIB) which showed totally positive expression when stained with this marker (figures 5&6).

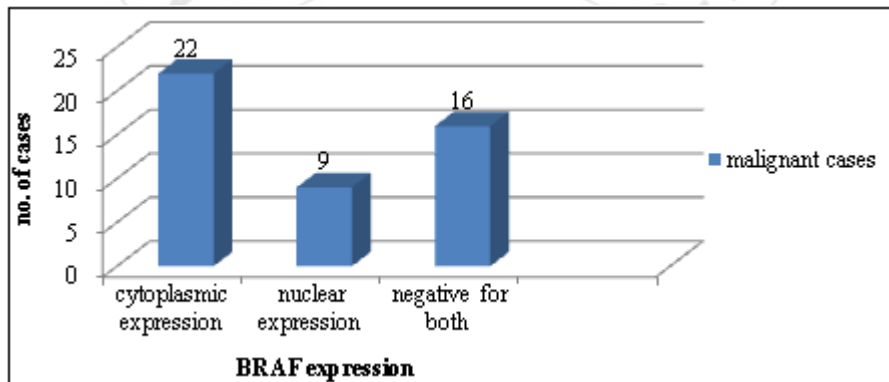


Figure 5: IHC Expression of BRAF in malignant group according to tumor stage

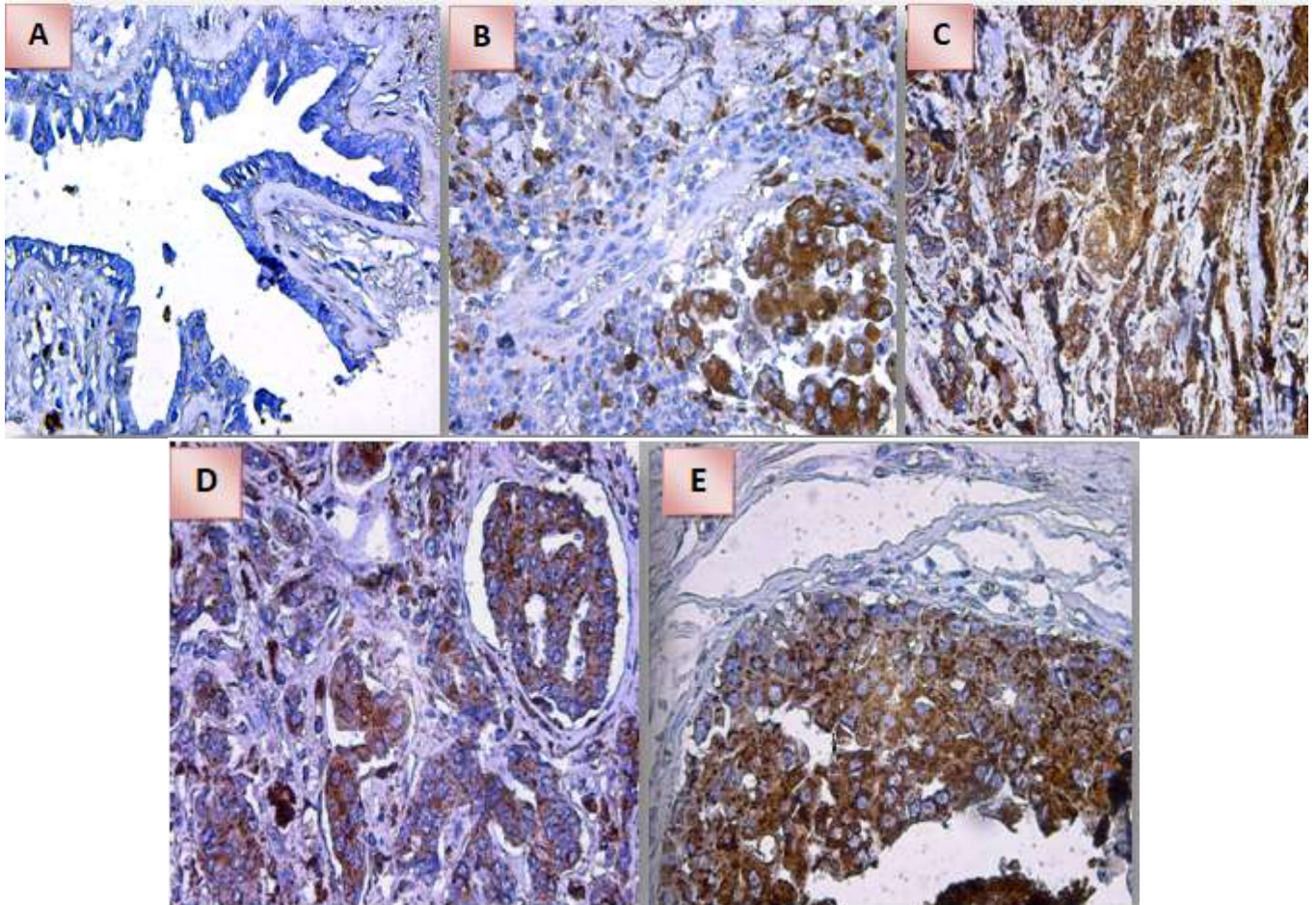


Figure 6: IHC expression of BRAF in different stages of IDC.

- A: IDC, Stage I, Negative expression of BRAF, 200x.
 - B: IDC, Stage IIA, positive expression of BRAF, 400x.
 - C: IDC, Stage IIB, positive expression of BRAF, 400x.
 - D: IDC, Stage IIIA, positive expression of BRAF, 400x.
 - E: IDC, Stage IIIB, positive expression of BRAF, 400x.
- IDC: Invasive Ductal Carcinoma

d) Immunohistochemical expression of BRAF according to tumor grade:

Results showed no significant correlation ($P=0.542$) between BRAF expression and tumor grade, that is, (3/6

)cases of grade I showed positivity to BRAF in comparison with grade II which showed positive result in(23/33) of cases and grade III in which BRAF was positive in(5/8)of cases (figures7&8).

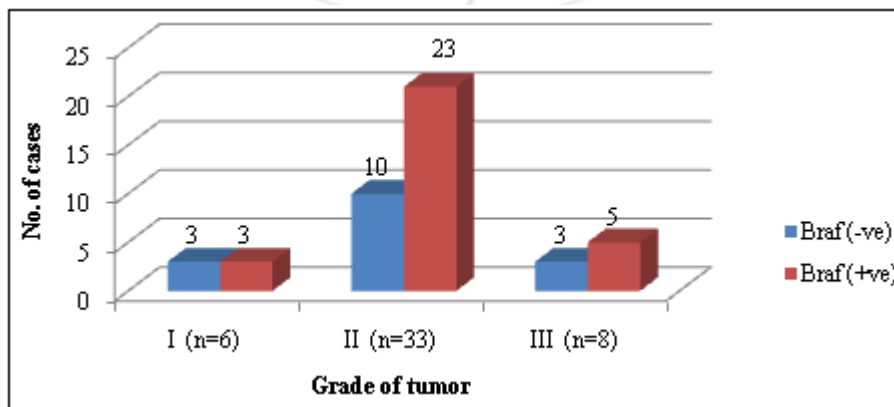


Figure 7: Expression of BRAF according to tumor grade

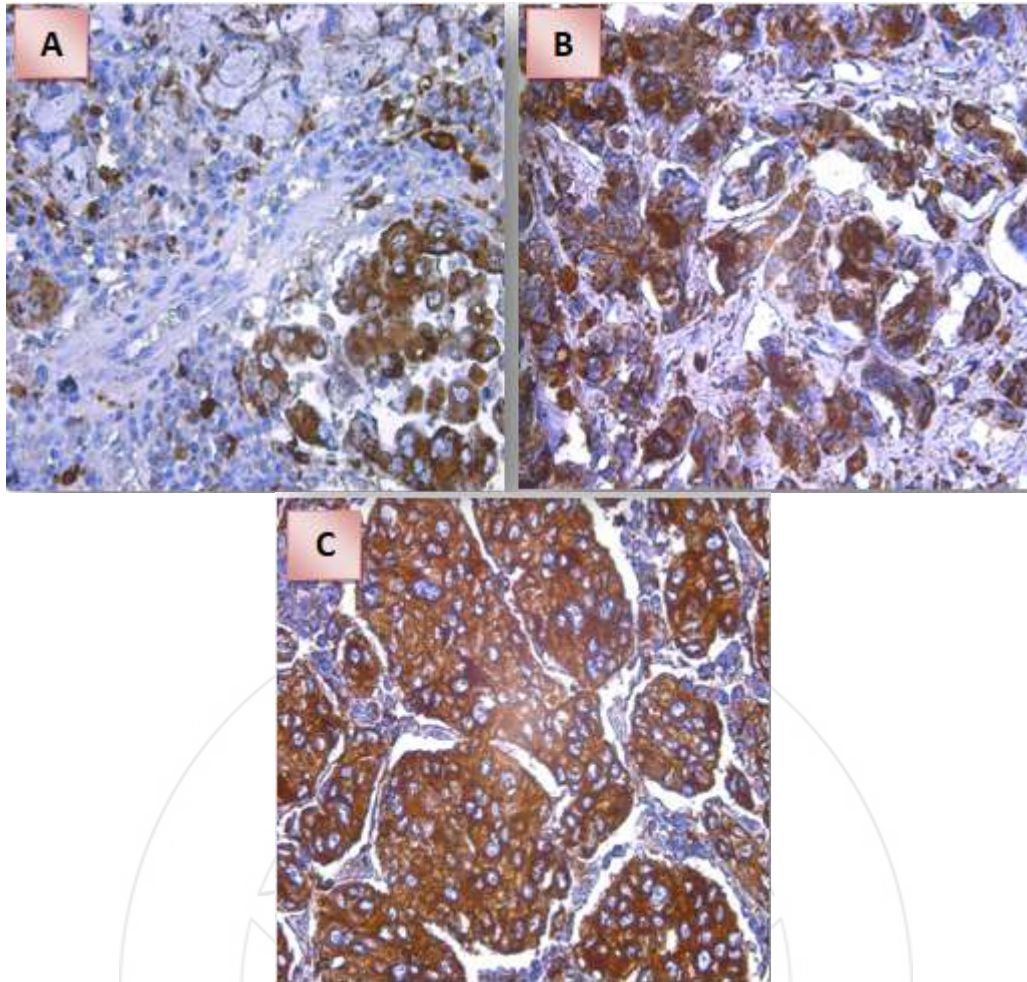


Figure 8: IHC expression of BRAF in different grades of IDC

(A) IDC, Grade I, positive expression IHC of BRAF, 400x, (B) IDC, Grade II, positive expression IHC of BRAF, 400 x, (C) IDC, Grade III, positive expression IHC of BRAF, 400 x, IDC: Invasive ductal carcinoma

4. Discussion

In this study, we examined pan BRAF status in breast cancer by IHC using BRAF specific antibody, and results revealed that 65.96% of breast cancer and 28.57% of benign tumors showed positivity for BRAF specific antibody. The typical method for the detection of BRAF mutations is Sanger sequencing analysis, and other PCR-based methods (11). But these methods need expensive tools, high technical skills, and have other problems such as tissue heterogeneity, sampling error, suboptimal DNA preservation of formalin-fixed paraffin-embedded (FFPE) tissue, and these drawbacks limited its' general use in clinical fields (13). To overcome these limitations, monoclonal specific anti-BRAF antibody was developed (14), and it is reported to be very useful in detecting BRAF mutation in various neoplasms(15). Especially in comparison studies using FFPE tissue, the IHC method using BRAF specific antibody was reported to be more specific and more sensitive compared to Sanger sequencing method, suggesting that IHC by BRAF specific antibody could be a new standard method (12). Consequently, our results of positive IHC staining of BRAF suggest that BRAF mutation is found in a proportion of breast cancers. In previous studies using sequencing methods, it is reported that 10% of breast cancer cell lines harbored BRAF mutation (9), 3% of breast cancer tissue had BRAF mutation, and BRAF

mutation was found in 2.6% in triple negative breast cancer [TNBC](11). Our study showed an elevated rate of BRAF mutation compared to previous studies regarding breast cancer. In previous studies regarding thyroid cancer tissues, the expression was reported to be cytoplasmic in positive cases in general (12, 16), while the result of our study with breast cancer showed diffuse cytoplasmic staining pattern in 71% of malignant cases, as well as nuclear expression to BRAF specific antibody that found in 29% of others. In previous studies regarding the expression of BRAF specific antibody in other neoplasms, only cytoplasmic expressions are reported (12, 14, 15, and 16). In this study, the BRAF status had significant association with age and tumor stage. In previous studies, BRAF expression is reported to be associated with several clinicopathologic features related to prognosis, such as lymph node metastasis and distant metastasis in PTC (17), younger age and tumor occurrence from intermittently sun-exposed skin in malignant melanoma (18, 19), poor prognosis in colon cancer (20, 21), which are agreed partially with the result from our study regarding breast cancer. The clinical implication of this study is the potential use of BRAF mutation status for the application of targeted therapy. In various neoplasms including malignant melanoma, drugs targeting BRAF mutation (vemurafenib and dabrafenib) is already in use in preclinical and clinical phase (22, 23), so targeted therapy in breast cancers with BRAF mutation can also be

considered. BRAF mutation targeted therapy can be a possible treatment, and a further study is needed.

5. Conclusion

In conclusion, a proportion of breast cancer showed positivity for the IHC by BRAF specific antibody, suggesting presence of BRAF mutation, and BRAF mutation show association with some clinicopathological factors.

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