

# BRAF mutation in hairy cell leukemia

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## Abstract

BRAF is a serine/threonine kinase with a regulatory role in the mitogen-activated protein kinase (MAPK) signaling pathway. A mutation in the *RAF* gene, especially in BRAF protein, leads to an increased stimulation of this cascade, causing uncontrolled cell division and development of malignancy. Several mutations have been observed in the gene coding for this protein in a variety of human malignancies, including hairy cell leukemia (HCL). BRAF V600E is the most common mutation reported in exon15 of BRAF, which is observed in almost all cases of classic HCL, but it is negative in other B-cell malignancies, including the HCL variant. Therefore it can be used as a marker to differentiate between these B-cell disorders. We also discuss the interaction between miRNAs and signaling pathways, including MAPK, in HCL. When this mutation is present, the use of BRAF protein inhibitors may represent an effective treatment. In this review we have evaluated the role of the mutation of the *BRAF* gene in the pathogenesis and progression of HCL.

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## Introduction

The mitogen-activated protein kinase (MAPK) is a pathway that regulates proliferation, differentiation, development, survival and apoptosis in response to growth factors, cytokines and hormones in mammalian cells.<sup>1,2</sup> Several protein kinases, including the BRAF protein, are involved in this pathway. BRAF is a serine/threonine kinase from the RAF kinase family encoded by the *BRAF* gene on chromosome 7 at 7q34 position, which has a regulatory role in the activation of MAPK/extracellular-signal-regulated kinase (ERK) signaling pathway (Figure 1A).<sup>3,4</sup> ERK/MAP kinase pathway is activated by a variety of receptors, including receptor tyrosine kinases and G-proteins, which, when stimulated, lead to the activation of the small G-protein of Ras.<sup>5</sup> Ras is an upstream activator of the Raf family of protein kinases (ARaf/BRaf/CRaf).<sup>6</sup> The three Raf kinases can all activate MEK1/2, which in turn activates ERK1/2, resulting in the phosphorylation of target proteins, including fos in, the nucleus and the transcription factors activator protein 1 (AP-1) and nuclear factor of activated T-cells (NFAT), which eventually lead to cell proliferation, differentiation and survival.<sup>1,7</sup> BRAF mutations directly cause activation of MEK and signal a transfer to ERK, leading to the activation of this pathway.<sup>8</sup> Excessive activation of this pathway leads to an uncontrolled cell cycle and plays an oncogenic role in human cancers.<sup>9</sup> Approximately 43 mutations have been identified in exons 11 and 15 of the *BRAF* gene, which are associated with a variety of human malignancies, the majority of which cause the activation of the kinase domain of this protein.<sup>10</sup> The most common BRAF mutation is V600E, which is caused by the substitution of adenine (A) for thymine (T) at position 1799 on exon 15 and results in the change of amino acid 600 from valine to glutamate in the protein sequence, followed by a constant activity of the downstream kinases, independently of the extracellular signals and increased cell proliferation (Figure 1B).<sup>11,12</sup> Hairy cell leukemia (HCL) has this type of pattern, and this mutation has been found in almost all classic cases of this malignancy.

HCL is a rare lymphoproliferative disorder of B-cells, and accounts for approximately 2% of leukemic diseases.<sup>13</sup> It is characterized by a progressive pancytopenia, splenomegaly without lymphadenopathy, presence of B cells with abnormal cytoplasm and a hairy look with infiltration in bone marrow, liver and spleen.<sup>14,15</sup> According to the 2008 World Health Organization (WHO) classification, diagnosis and differentiation of HCL from similar diseases, including splenic marginal zone lymphoma (SMZL), splenic lymphoma/leukemia unclassifiable, such as HCL variant (HCL-v), are based on morphological and immunophenotypic markers.<sup>14</sup> Malignant cells co-express CD20, CD22, CD11c, CD25, CD103, tartrate-resistant acid phosphatase and Anxin A1.<sup>16,17</sup> Classic HCL responds to purine nucleoside analogs, while HCL-v cases are resistant and are more aggressive compared with the classic variant.<sup>18</sup> Therefore distinguishing classic HCL from

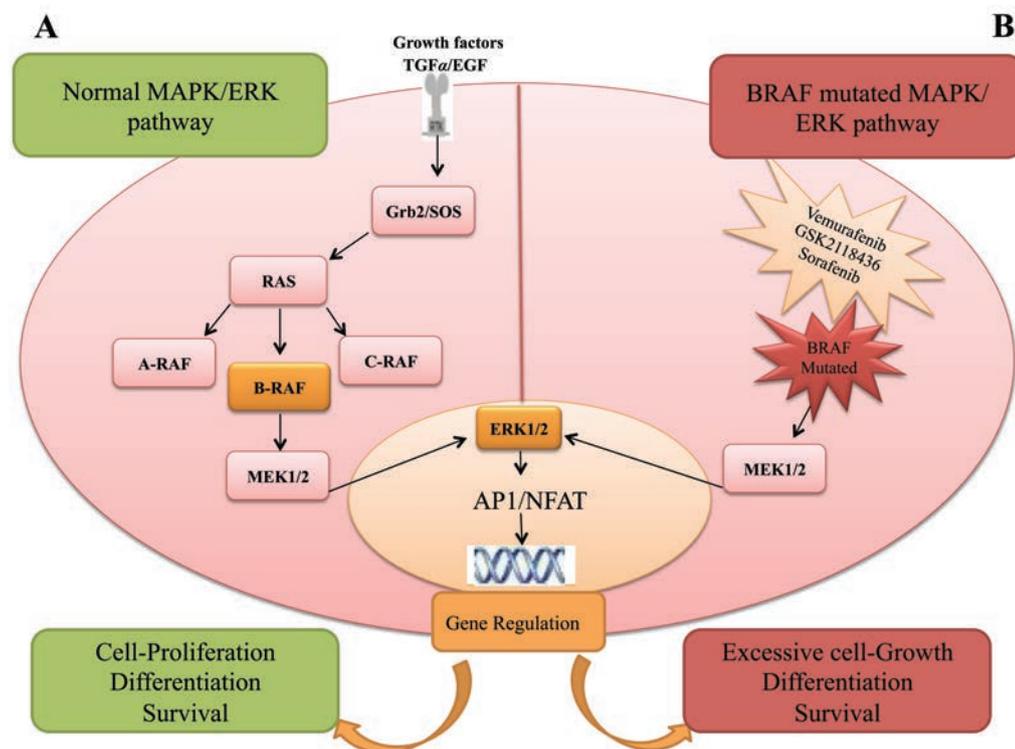
other B-cell lymphomas, including HCL-v and SMZL, is critical, since treatment with purine nucleoside analogues is only effective in HCL. In this review, the association between HCL and BRAF mutation as well as miRNA will be discussed. We also show that targeting the BRAF oncogene with inhibitors provides a new therapeutic strategy, and BRAF mutation testing can be used for laboratory diagnosis of HCL.

## BRAF mutation and hairy cell leukemia

Increased activity of MAPK pathway has been reported in a small number of patients with HCL, and plays a role in the growth and survival of HCL cells.<sup>19</sup> The presence of BRAF V600E mutation leads to increased phosphorylation of MEK and ERK, followed by increased phosphorylation of AP-1 and NFAT transcription factors.<sup>1,7</sup> AP-1 activation has been associated with the expression of HCL marker CD11c.<sup>20</sup> Tiacci *et al.* found the BRAF V600E mutation in exon 15 of *BRAF* gene in 100% of classic HCL cases (n=48) using Sanger sequencing, but not in other B-cell lymphomas/leukemias.<sup>21</sup> Blombery *et al.* in their study confirmed the detection of BRAF V600E mutation in all classic HCL patients. In addition, they observed BRAF V600E mutation in patients with morphologically classic HCL with immunophenotypic variations (CD25<sup>+</sup>, CD10<sup>+</sup>, CD123<sup>+</sup>).<sup>22</sup> In addition, in a study by Arcaini *et al.*, BRAF V600E mutation was detected in all 62 HCL patients and only in 2 patients with other hematological malignancies.<sup>23</sup> This mutation is not detected in HCL-v and IGHV4-34 variants of HCL.<sup>24</sup> These findings confirmed that BRAF mutation V600E is a molecular hallmark of HCL. Two novel mutations in exon 11 of *BRAF* gene were detected in BRAF V600E-negative HCLs.<sup>25</sup> It has been suggested to screen exon 11 in V600E-negative HCL cases. BRAF mutation was present in hematopoietic stem cells in patients with HCL.<sup>26</sup>

## Hairy cell leukemia and miRNA

MicroRNAs are small 19-22 nucleotide RNA molecules involved in the regulation of processes such as proliferation, apoptosis and suppression of target gene expression.<sup>27,28</sup> MiRNA expression is altered in cancer.<sup>29</sup> BRAF mutation may lead to a changing expression of several miRNAs with regulatory roles for several oncogenes, including BRAF. In recent years, the relationship between miRNA and a mutation in the *BRAF* gene with a number of human cancers including papillary thyroid carcinoma (PTC) has been recognized. It was demonstrated that the expression of miR-221, miR-146b and miR-222 was higher in patients with BRAF mutation, and the invasiveness and the metastasis were more severe among them.<sup>30</sup> In 2012, Zhou *et al.* showed that the expression level of miRNA-221 was higher in PTC patients with a mutation in *BRAF* gene, which can be involved in the extra-thyroid invasion of the tumor, tumor size and disease progression rate.<sup>31</sup> The contribution of miRNAs to HCL has been analyzed by Kitagawa *et al.* in 2012. They identified miRNA, -221/-222 family, -22, -24, 27a and Let-7b in a higher level of expression in HCLs compared with normal and other malignant B-cells.<sup>32</sup> They also suggested that miR-221/-222 overexpression in HCL may lead to a low expression of CDKN1B (p27/Kip1), which is a direct target of these miRNAs.<sup>33</sup> On the basis of the association between BRAF mutation and miR-221/-222 in PTC,<sup>30</sup> a high level of this miRNA in HCL suggests that miR-221/-222 overexpression in HCL regulates MAPK signaling in HCL, and correlates with BRAF mutation. In chronic lymphocytic leukemia (CLL), cell proliferation is due to the overexpression of miR-22, which induces phosphatase and tensin analog down regulation and PI3-Akt pathway activation.<sup>34</sup> In addition there is an upregulation in Cyclin D2 (*CCND2*) and *MAPK1* genes.<sup>34</sup> As a consequence, we can suggest that miR-22 may modulate MAPK signaling in HCL and requires further investigation. MiR-24 activates



**Figure 1.** Role of BRAF in (A) normal and (B) mutated state in the activation of MAPK pathway. MAPK inhibitor drugs, such as vemurafenib, inhibit this protein by binding the mutated BRAF molecule, thus halting its activity and an excessive activation of the MAPK pathway.

MAPK signaling by downregulating MAPK phosphatase 7 in acute myeloid leukemia.<sup>35</sup> Therefore miR-24 is also related to MAPK signaling and can contribute to BRAF mutation in HCL. In the study of Moussay *et al.*, the level of miR-363 and miR-708 was lower and higher in HCL and CLL, respectively. Also, there were no significant changes in miR-34a and miR-564 in HCL compared with normal subjects.<sup>36</sup> MiRNA deregulation may be associated with prognosis and disease progression in HCL. The combined evaluation of the HCL-specific miRNA signature and its association with MAPK signaling may be useful to better understand the pathogenesis and management of HCL.

## Treatment and control of mutant BRAF effects

Due to the importance of Ras and BRAF in the development of malignancies, Raf inhibitors have been generated especially against BRAF V600E.<sup>37</sup> BRAF inhibition results in cell death by suppressing the Ras/Raf/MEK/ERK pathway in HCL.<sup>38</sup> In *in vitro* studies, it was demonstrated that HCL cells undergo apoptosis with BRAF inhibitors.<sup>39</sup> Vemurafenib, which inhibits thymidine kinase enzyme, has been recently approved for the treatment of patients with metastatic melanoma with BRAF V600E mutation after a randomized phase III trial. The overall survival and progression-free survival was improved in this group of patients.<sup>40</sup> Dietrich *et al.* confirmed the mutant BRAF as a therapeutic target in HCL by using vemurafenib in patient with refractory HCL.<sup>41,42</sup> Vemurafenib led to complete clinical remission by reducing the viability of CD-103<sup>+</sup> HCL cells.<sup>38</sup> Other inhibitor compounds are also currently used in treatment of HCL (Table 1),<sup>43,44</sup> which mainly bind the BRAF molecule and prevent MEK and ERK phosphorylation and cell division. The above-mentioned material validates the mutant BRAF as a therapeutic target in HCL.

## Discussion

*BRAF* is a commonly mutated gene in a variety of cancers.<sup>9</sup> BRAF V600E mutation occurs in 40% to 70% of malignant melanomas, 45% of PTC and 5-15% of colorectal cancers, 35% of ovarian tumors and 1-3% of cases of non-small cell lung carcinoma.<sup>45,46</sup> The most common BRAF mutation is BRAF V600E, which results in the constant activity of downstream kinases, independently of extracellular signals and increased cell proliferation.<sup>11</sup> In hematological malignancies, BRAF V600E has been defined as a genetic lesion in HCL.<sup>21</sup> Until now, almost all cases of HCL (100%) show this mutation. However, BRAF V600E mutation is not detected in other B-cell malignancies, including SMZL, HCL-v, CLL, mantle cell lymphoma and Waldenstrom macroglobulinemia.<sup>21,47</sup> Therefore, BRAF V600E mutation may be a hallmark of HCL. Regulation

**Table 1. Binding site of a number of inhibitor drugs of the MAPK pathway.**

Drug (inhibitors)	Binding site	Ref
Vemurafenib or (PLX4032, PG7204, RO5185436)	BRAF V600E	43
Sorafenib	RAF	43
GSK2118436	RAF	43
AZD6244	MEK	43
GSK1120212	MEK	43
SB590885	BRAF	44

of the MAPK pathway by a specific miRNA signature has been recently reported in HCL by Kitagawa *et al.*<sup>32</sup> A deeper understanding of the relationship between miRNA and signaling pathways like MAPK may improve our knowledge of HCL pathogenesis. The function of miRNAs in HCL and their association with the MAPK pathway needs further investigation, as it may be used as a biomarker or regulator and enable clinical applications in HCL.

The use of inhibitor drugs that specifically bind to mutant BRAF molecules and halt the continuation of the pathway is effective in preventing disease progression. The clinical efficacy of BRAF kinase inhibitors has been reported in melanoma.<sup>48,49</sup> BRAF inhibition discontinues the constant activation of the MAPK pathway, thus offering a new therapeutic opportunity in the treatment of BRAF V600E refractory HCL.<sup>36</sup> Dietrich *et al.* confirmed the role of mutant BRAF as therapeutic target in HCL, using vemurafenib in patients with refractory HCL.<sup>41,42</sup>

We can therefore conclude that BRAF V600E mutation, which has been found in almost all cases of classic HCL, can be used as a marker to differentiate this disease from similar diseases, such as HCL-v. However the evaluation of the BRAF mutation is not a routine clinical test for HCL, but molecular detection of BRAF V600E may be helpful in the diagnosis of HCL patients and in their follow up after treatment in order to monitor the disease and detect relapse. Further studies are required to establish the clinical benefit of this mutation in HCL.

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