**Short Communication**

**BRAF mutations in non-Hodgkin’s lymphoma**

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Ras proteins control signalling pathways that are key regulators of several aspects of normal cell growth and malignant transformation. BRAF, which encodes an RAF family member in the downstream pathway of RAS, is somatically mutated in a number of human cancers. The activating mutation of BRAF is known to play a role in tumour development. As there have been no data on the BRAF mutation in non-Hodgkin’s lymphoma (NHL), we analysed the genomic DNAs from 164 NHLs by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) for the detection of somatic mutations of BRAF (exons 11 and 15). Overall, we detected BRAF mutations in four NHLs (2.4%). Whereas most BRAF mutations in human cancers involved V599 of BRAF, all of the four BRAF mutations in the NHLs involved other amino acids (one G468A, two G468R and one D593G). To our knowledge, this is the first report on BRAF mutation in NHL, and the data indicate that BRAF is occasionally mutated in NHL, and suggest that BRAF mutation may contribute to the tumour development in some NHLs.

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**MATERIALS AND METHODS**

Paraffin-embedded tissues of human NHL were obtained from 164 patients. These samples were stained with haematoxylin–eosin, examined by immunohistology, and then classified according to the Revised European-American Lymphoma (REAL) classification (Harris et al, 1994). The NHLs analysed consisted of seven B-cell small lymphocytic lymphomas, three mantle cell lymphomas, four follicular lymphomas, 49 mucosa-associated lymphoid tissue (MALT)-type lymphomas, 67 diffuse large B-cell lymphomas, four precursor T-lymphoblastic lymphomas, one T-cell chronic lymphocytic leukaemia, 14 peripheral T-cell lymphomas, unclassified, 14 angiocentric lymphomas, and one intestinal T-cell lymphoma. Ethical committee approval for the study was obtained. Through the microdissection technique, we selectively procured tumour cells and corresponding normal cells from histological sections of the 164 NHLs. Briefly, malignant cells were selectively procured from haematoxylin and eosin-stained sections using a 30G1/2 hypodermic needle (Becton Dickinson, Franklin Lakes, NJ, USA) affixed to a micromanipulator, as described previously (Lee et al, 1998). We also microdissected normal cells and used them for corresponding normal DNA. This microdissection technique used in this study has been proved to be precise and effective for procurement of tumour cells without normal cell contamination (Lee et al, 1998). DNA extraction was performed by a modified single-step DNA extraction method, as described previously (Lee et al, 1998).

Genomic DNA each from normal cells or tumour cells was amplified with two primer pairs covering exons 11 and 15 of BRAF gene, because all of the BRAF mutations have been so far detected in exons 11 and 15 that encode the kinase domains in G-loop and the activation segment of BRAF, respectively. Radioisotope was incorporated into the PCR products for detection by autoradiogram. The PCR reaction mixture was denatured for 1 min at 94°C.

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and incubated for 30 cycles. Other procedures of polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) analysis were performed as described previously (Shin et al., 1999). After SSCP, DNAs showing mobility shifts were cutout from the dried gel, and reamplified for 30 cycles using the same primer sets. Sequencing of the PCR products was carried out using the cyclic sequencing kit (Perkin-Elmer, Foster City, CA, USA) according to the manufacturer’s recommendation.

RESULTS

SSCP analysis of BRAF identified four aberrant bands (Figure 1). Enrichment and DNA sequence analysis of these aberrantly migrating bands led to the identification of four BRAF mutations (2.4%) (Figure 1). All of the four BRAF mutations were observed in diffuse large B-cell lymphomas (6.0% of the 67 cases). Although BRAF mutations were detected only in diffuse large B-cell lymphomas, this relationship was not statistically significant (P > 0.05). Three of the four BRAF mutations involved codon 468 (two G468R and one G468A) in the G-loop domain, and the remaining one was found at codon 593 (D593G) in the activation segment domain (Table 1, Figure 1). None of the corresponding normal samples showed evidence of mutations by SSCP (Figure 1), indicating the mutations detected in the specimens had risen somatically. We repeated the experiments two times, including tissue microdissection, PCR, SSCP and sequencing analysis to ensure the specificity of the results, and found that the data were consistent (data not shown).

DISCUSSION

Whereas the malignant melanoma is the most common tumour with BRAF mutations (roughly 60%), this tumour is known to possess a much lesser frequency of RAS mutations. Such differential occurrences of BRAF and RAS mutation in some human cancers led us to analyse BRAF mutation in NHL in which RAS mutation is known to be an uncommon event. We found that BRAF gene is somatically mutated in NHLs, indicating that RAS–RAF kinase pathway in some NHLs may be regulated by somatic mutations of BRAF. Despite the low frequency of BRAF mutation in NHL compared with that of malignant melanoma, our data suggest that alteration of RAS–RAF kinase pathway by BRAF mutation may play an important role in NHL carcinogenesis.

In the present study, none of the BRAF mutations involved the amino acid V599. The data are quite contrast to those of malignant melanomas, where approximately 90% of BRAF mutations involved V599, raising the possibility that the contribution of BRAF mutations in the development of NHL might be different from that of malignant melanoma. Additionally, three (two G468A and one G468R) of the four BRAF mutations in this study involved the same amino acid (G468) that is located in the GXGXXG motif within the G-loop of the kinase domain. The G468A mutation was proven to be an activating mutation by the kinase assay and the transformation assay (Davies et al., 2002). For G468R, a novel BRAF mutation, its functional implication is not known at this time.
stage. In one NHL, we also found D593G BRAF mutation that has also been detected in colon tumours previously.

The most impressive examples of recent cancer therapies used protein kinase inhibitors such as Imanitib (Gleevec) (Downward, 2003). Since RAS – RAF – MEK – ERK – MAP kinase pathway is activated by protein kinase, therapies that target this signalling pathway would therefore be very valuable in treating tumours that have activating mutations of BRAF. In this respect, the present study may provide the possibility of therapy targeting mutated BRAF in NHL.

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Table 1 Summary of BRAF mutations identified in the NHLs

| Nucleotide | Amino acids | Anatomical site | Histologic type |
|------------|------------|----------------|----------------|
| G1403C     | G468A      | Cervical lymph node | Diffuse large B-cell lymphoma |
| G1403C     | G468A      | Cervical lymph node | Diffuse large B-cell lymphoma |
| G1402C     | G468R      | Tongue mucosa    | Diffuse large B-cell lymphoma |
| A1778G     | D593G      | Ileum           | Diffuse large B-cell lymphoma |

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