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*NF*1 Gene Mutation and Acute Myelogenous Leukaemia

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WE READ with interest the report by Tenan and colleagues in the recent issue of the *European Journal of Cancer* [1] on the low frequency of *NF*1 gene mutation in malignant gliomas. We have performed a similar study concerning the mutation of *NF*1 gene in acute myelogenous leukaemia (AML).

*NF*1 gene is responsible for von Recklinghausen's neurofibromatosis (neurofibromatosis type 1: NF1), which is an autosomal dominant disease associated with an increased risk of benign and malignant neoplasms [2]. The product of *NF*1 gene, neurofibromin, contains a domain, structurally and functionally homologous to GTPase activating protein (GAP), which negatively regulates the *ras* oncogene product ($p21^{ras}$) [2]. Since activated $p21^{ras}$ has been found in human tumours, and implicated in the pathogenesis of many cancers [3], the *NF*1 gene is considered to be a tumour suppressor gene.

Alterations of the first nucleotide position of the Lys-1423 codon, which results in the amino acid change and the loss of GAP activity of the mutant neurofibromin, have been reported in three tumour types: colon adenocarcinoma, anaplastic astrocytoma and myelodysplastic syndrome (1/28 patients) [4]. Considering that mutational activation of *ras* is found in approximately one third of myelodysplastic syndrome (preleukaemia) and AML patients [3], there is also a possibility that *ras* activation, through impaired negative regulation by mutated neurofibromin, is involved in the development of these two myeloid disorders.

In addition to the first nucleotide position of Lys-1423 codon (AAG) in exon 24, as described above, we and others [5–8] identified another hotspot in NF1 patients. The first nucleotide position of Arg-1947 codon (CGA) in exon 31 is converted to T, which results in the generation of a stop codon (TGA), thereby approximately one third of neurofibromin is not translated.

In order to clarify the role of *ras* activation in AML, we studied 23 AML patients (3 patients of M1, 15 patients of M2, 2 patients of M3 and 3 patients of M4) for the mutations at these two hotspots in the *NF*1 gene.

DNA samples were prepared from peripheral blood mononuclear cells (containing 50–90% of blasts) from patients, and analysed by polymerase chain reaction using single strand conformation polymorphism analysis (PCR-SSCP) method [9], using intron-based primer pairs [4, 5] for amplifying exons 24 and 31, which contain the hotspots. The PCR-SSCP analysis did not reveal any band of altered mobility, suggesting that there were no mutations in either of the hotspots in the *NF*1 gene in our 23 AML patients.

Although we investigated only a limited region of the large *NF*1 gene, and still have to expand the number of patients, both of the hotspots so far identified in *NF*1 gene were not mutated in our AML patients. Therefore, we suggest that *NF*1 gene mutation does not play an important role in the pathogenesis of AML.

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High Cell Proliferation Activity Determined by DNA Flow Cytometry Predicts Poor Prognosis After Relapse in Prostate Cancer

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SEVERAL STUDIES have shown that DNA aneuploidy and/or high cell proliferation activity determined by DNA flow cytometry is

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