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A new minimal deleted region at 11q22.3 reveals the importance of interpretation of diminished FISH signals and the choice of probe for *ATM* deletion screening in chronic lymphocytic leukemia

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ABSTRACT

Deletion of ATM detected by fluorescent in situ hybridization (FISH) in chronic lymphocytic leukemia predicts short treatment free survival and poor outcome following alkylator/purine analogue therapy. We describe five cases, with a diminished ATM FISH signal, investigated by *TP53* mutation/dysfunction studies and single nucleotide polymorphism (SNP) array. The diminished signal represented loss of the *ATM* gene, which could have been missed were the cases not further investigated. These rare cases highlight the need for careful consideration of the choice of probe and interpretation of unusual signal patterns in FISH screening. We define a new minimal region of deletion at 11q22.3.

1. Introduction

Deletion of chromosome 11 involving bands q22–23 was initially found as a recurrent abnormality in CLL by conventional karyotype analysis, in approximately 9% of cases depending on the cohort examined [1,2]. However, such deletions are heterogeneous in size and when ascertainment is by FISH with probes from the q22–23 region, the frequency of their detection increases to 10–20%. FISH mapping studies have defined a minimal region of deletion (MDR) of 2–3 Mb that includes the *ATM* gene [3]. Deletion of this region, detected by FISH, predicts for short treatment free survival and poor outcome following alkylator/purine analogue therapy [4], although, in the German CLL8 trial, addition of Rituximab lead to improved outcomes [5]. In a routine clinical setting FISH is used as a proven, well-established method to identify CLL patients with 11q22.3 loss. Recently newer methodologies, with higher resolution, such as array based comparative genomic hybridization, SNP arrays and representational oligonucleotide array analysis have been used to examine genomic abnormality in CLL in a number of studies, determining regions of copy number variation (CNV) and loss of heterozygosity [6–8]. For the most part these studies have investigated small cohorts of patients and have confirmed but not refined the MDR at 11q23 established by FISH. However, a recent study using SNP analysis has shown a MDR of 0.68Mbp at 11q22.3 which includes the *RAB39*, *CUL5*, *ACAT1*, *NPAT* and *KDELC* genes [9].

During routine FISH screening we have identified 5 cases with a diminished ATM probe signal when hybridised with a widely used commercial probe. In this study we have investigated these cases further using a variety of techniques to determine the significance of this unusual finding.

2. Methods and patients

2.1. Patients

We have screened 849 CLL patients, by interphase FISH, for loss of *ATM*. 80% were tested at presentation, while the remainder were tested during the disease course, usually prior to treatment. This study, initiated as a result of routine FISH screening, was approved by the Somerset Research Ethics Committee.

2.2. FISH and cytogenetics

Cells for FISH were prepared from peripheral blood by density centrifugation using Histopaque™ (Sigma, UK). The resultant mononuclear cells were then

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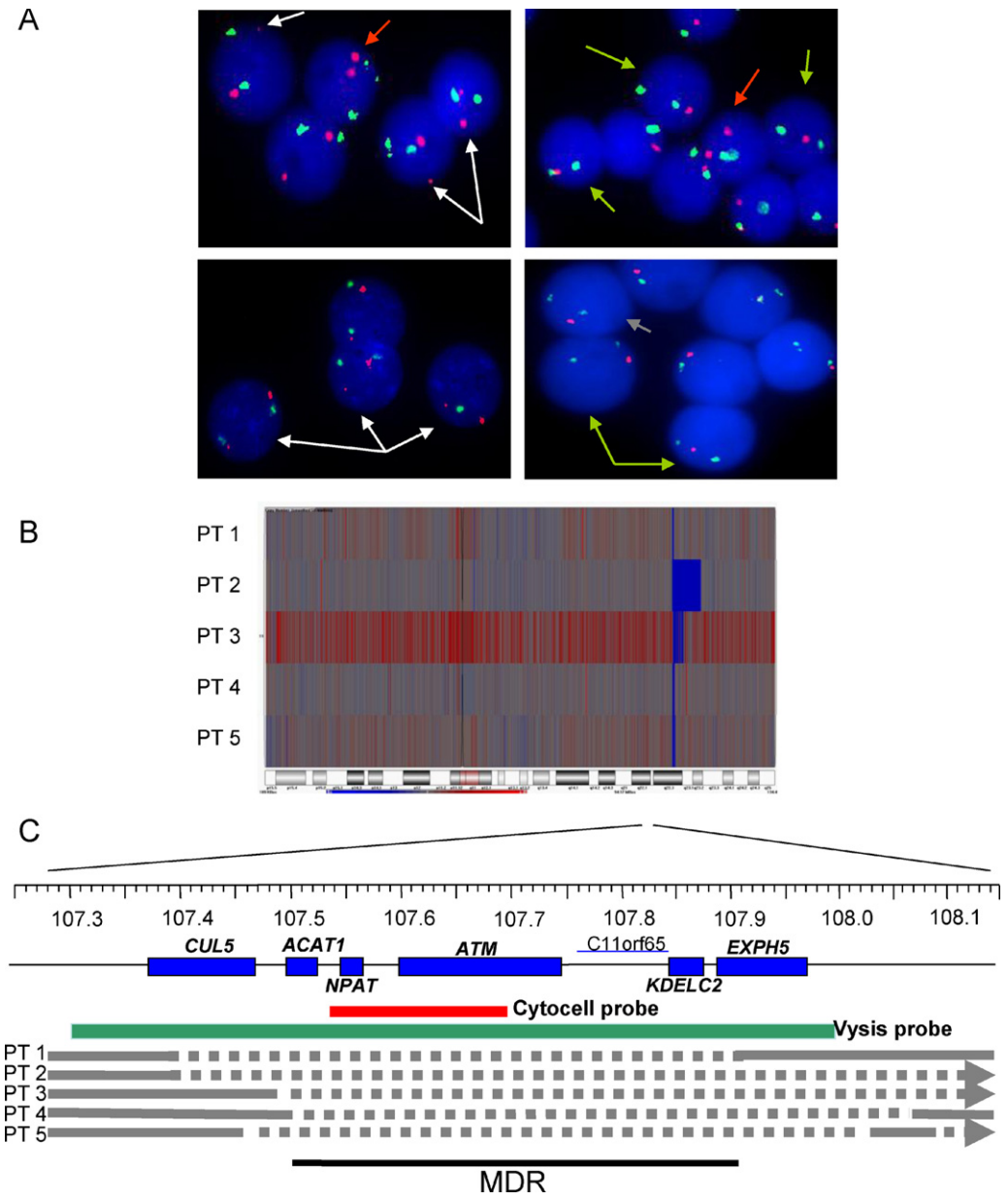

Fig. 1. Hybridization images and schematic representation of 11q23 region. A: nuclei from 2 patients, Vysis probes – right, Cytocell probes – left. Arrows: white = diminished ATM(red) signals, orange = normal, green = 1xATM loss. B: chromosome 11 heatmaps, blue = loss. C: dotted lines = deleted region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

treated with hypotonic solution (0.075 M KCl) and fixed with methanol/acetic acid (3:1) prior to slide preparation, using 2 μl of cell suspension. Initially, interphase FISH for loss of *ATM* was undertaken with a widely used commercial probe, that recognises a >500 kb region encompassing both *ATM* and flanking genes, which was co hybridized with a chromosome 11 specific centromere probe (Vysis LSI® ATM-11q22.3 SO and CEP11 Alpha SG, Abbott Laboratories, UK). Both interphase and metaphase preparations from subsets of cases were subsequently tested with an ATM FISH probe mix, consisting of a smaller ATM probe (158 kb) and a control probe for 11 centromere (Cytocell Aquarius ATM deletion probe, Labtech International Ltd.). Hybridization with both Cytocell and Vysis probes was undertaken using the same fixed cell suspension from each patient. In all instances hybridization was according to the manufacturer's protocol. Images were captured by a Hamamatsu digital camera using SmartCapture 3 software (Digital scientific, Cambridge) and a Zeiss Axioscop microscope fitted with epifluorescence. The spatial relationship of the two probes to the *ATM* gene is shown schematically in Fig. 1.

For 4 cases with a diminished ATM probe signal, G banded metaphase analysis was also available from TPA (Phorbol 12-myristate 13-acetate, Sigma–Aldrich, USA) stimulated cultures. Resulting karyotypes are described according to ISCN [10].

2.3. SNP analysis

Tumour DNA was amplified, labelled and hybridised to the Affymetrix SNP 6.0 platform, according to the Affymetrix Cytogenetics Copy Number protocol (Affymetrix, Santa Clara, CA, USA). The SNP 6.0 array includes 1.8 million probes with an average inter-marker distance of 700 kb, optimized for SNP and copy number changes. Results for copy number were interpreted using the Partek software suite (Partek Inc., Missouri, USA). All gene and probe locations are given according to USCS Genome browser – March 2006 Assembly.

2.4. ATM mutation and functional analysis

Evaluation of the mutational status of the *ATM* gene was determined using a denaturing high performance liquid chromatography (HPLC) and sequencing approach covering 62 coding exons [11]. Functional evidence for an *ATM* mutation was sought using an assay in which leukemic cells were exposed to etoposide and nutlin-3a and subsequently p53 and p21 expression were measured by flow cytometry. In this assay patients with *ATM* mutation frequently show attenuated levels of p53 and p21 expression following exposure to etoposide alone but show a normal response to etoposide and nutlin; this pattern is designated Type 2 response [12].