

Detection of t(7;12)(q36;p13) in paediatric leukaemia using dual colour fluorescence in situ hybridisation

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Abstract

The identification of chromosomal rearrangements is of utmost importance for the diagnosis and classification of specific leukaemia subtypes and therefore has an impact on therapy choices in individual cases. The t(7;12)(q36;p13) is a cryptic rearrangement that is difficult to recognise using conventional cytogenetic methods and is often undetected by reverse transcription polymerase chain reaction due to the absence of a fusion transcript in many cases. Here we present a reliable and easy to use dual colour fluorescence in situ hybridisation assay for the detection of the t(7;12)(q36;p13) rearrangement. A comparison with previous similar work is given and advantages and limitations of this novel approach are discussed.

Keywords: Fluorescence in situ hybridisation, t(7;12), HLXB9, ETV6, chromosome abnormalities, myeloid malignancies, paediatric leukaemia

Introduction

Chromosomal translocations are a hallmark of cancer and leukaemia and are useful biomarkers for disease diagnosis and follow up [1]. The t(7;12)(q36;p13) rearrangement is a rare chromosomal translocation recurrently found in a subset of paediatric leukaemia affecting mostly children between 0-2 years, also known as infant leukaemia [2-5]. It has been estimated that this cytogenetic abnormality occurs in approximately one third of infant leukaemia, representing the second most common rearrangement after those involving the *MLL* gene on 11q23 [2,6]. Conventional cytogenetic methods of chromosome banding are broadly used in diagnostic laboratories. However, these have limitations when analysing complex or cryptic abnormalities. Because the t(7;12)(q36;p13) affects the terminal regions of 7q and 12p respectively, the rearrangement is difficult to identify by conventional karyotyping. Nowadays, various other techniques are in place for the detection of chromosomal translocations. One of these is reverse transcription polymerase chain reaction (RT-PCR) that uses primers specific for known translocations, identifiable when a fusion transcript is present [7]. This approach is commonly used in diagnostic laboratories as it allows for a quick detection of the abnormalities. A fusion transcript arising from the t(7;12) rearrangement has been described as the HLXB9-ETV6 fusion [8]. However,

this fusion transcript has been identified in only approximately 50% of cases harbouring the t(7;12)(q36;p13) [6,9]. Therefore, a more reliable method must be used for the detection of this rearrangement. Fluorescence in situ hybridisation (FISH) is currently applied in many diagnostic laboratories using in-house made probes, custom made probes or commercially available probes. An advantage of using FISH is the possibility of conducting single cell analysis on both metaphase chromosomes and interphase nuclei, allowing the possibility to estimate the proportion of cells carrying the abnormality. Here we report the use of a novel dual colour FISH assay for the identification of the t(7;12)(q36;p13) rearrangement in paediatric leukaemia. This has been designed as an alternative to a previously described three colour probe set [10].

Findings

Patient samples

Archival methanol: acetic acid fixed chromosomes and cells suspensions from four patients previously described were used in this study [2,10-12]. All of the patients were children affected by myeloid disorders (see Table 1). The samples were selected on the basis of harbouring a t(7;12) rearrangement previously characterised using FISH assays different from the one here reported.

Table 1. Clinical features, cytogenetic data and FISH findings relative to the patients analysed in this study.

pt	Diagnosis		Previous FISH probes	Reference	Comparison with previous FISH studies
1	T-ALL later revised as AML	49,XX,+6,t(7;12)(q36;p13),+8,+19[3]/ 50,idem,+18[7]	YAC 964c10 (12p13) Vysis TEL/AML1 ETV6 exon specific cosmids YAC HSC7E551 (7q35) YAC HSC7E526 (7q36)	pt. 4 in [2]	Refined chromosome 7 breakpoint
2	AML-M4	48,XY, t(7;12)(q36;p13),+8,+19	YAC 964c10 (12p13) ETV6 exon specific cosmids PAC H_DJ1121A15 (7q36) Cos 121b1 (7q36) Cos 227e6 (7q36) Cos 85c10 (7q36) Cos 133f02 (7q36)	pt 6 in [2] pt 4 in [12]	Confirmed previous findings
3	AML-M0	47,XY,t(7;12)(q36;p13),+der(19)	YAC 964c10 (12p13) ETV6 exon specific cosmids PAC H_DJ1121A15 (7q36) Cos 121b1 (7q36) Cos 227e6 (7q36) Cos 107a07 (7q36) Cos 148a04 (7q36) Cos 85c10 (7q36) Cos 133f02 (7q36) Three colour FISH probe	pt 5 in [2] pt 3 in [12] Pt 2 in [10]	Confirmed previous findings
4	MDS	46,XX,der(7)t(7;12)(q22;p13)del(7) (q22q36)	YAC 964c10 (12p13) ETV6 exon specific cosmids Various 7q22and 7q36 probes Three colour FISH probe	pt 1 in [2] pt 1 in [12] pt 1 in [10]	Confirmed previous findings

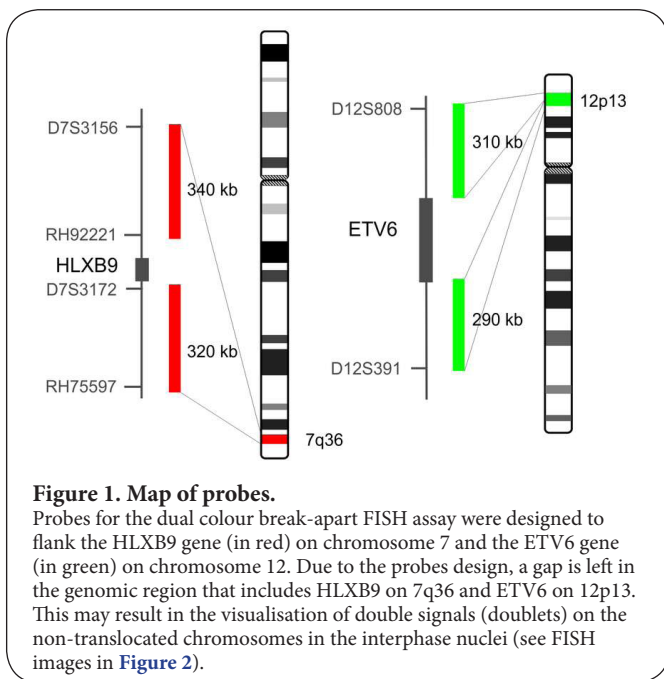
Fluorescence in situ hybridization (FISH)

A dual colour FISH probe set for the detection of the t(7;12)(q36;p13) was provided by MetaSystems (MetaSystems GmbH, Altlußheim, Germany). A schematic representation of the probes and their location along chromosomes 7 and 12 is shown in **Figure 1**. FISH experiments were carried out according to the manufacturer's instructions with slight modifications. Briefly: slides were denatured with 70% formamide at 72°C for 3 min. Probe mixture was denatured at 72°C for 8 min, incubated at 37°C for 30 min, and subsequently applied to the slides under a 22×22 mm cover-slip. After overnight hybridization, slides were washed with 2×SSC (pH 7.0) for 5 min on shaker, then transferred to 0.4×SSC (pH 7.0) at 70°C for 5 min followed by another wash in 2×SSC/Tween20 for 5 min on shaker and then a final wash in 1×PBS for 5 min on shaker. The slides were then mounted in Vectashield

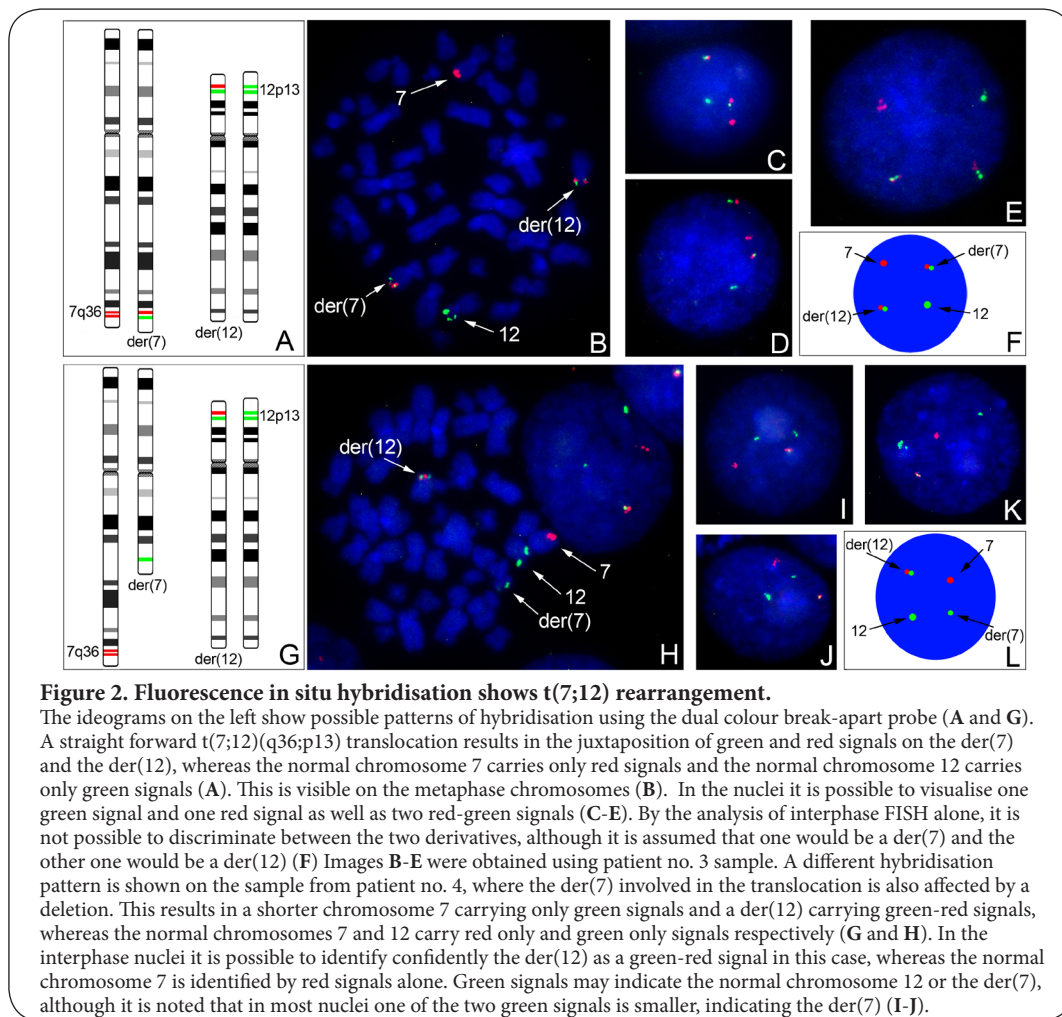
(Vector Laboratories Ltd., Peterborough, UK) containing 49, 6-Diamidine-29-phenylindole dihydrochloride (DAPI). Hybridized chromosomes and nuclei were viewed and images were captured using an Olympus BX41 fluorescence microscope and SmartCapture 3 software (Digital Scientific, Cambridge, UK).

Results and discussion

The dual colour FISH approach enabled us to detect the t(7;12) rearrangement in all four cases analysed. In three of the patient samples (patient nos. 1-3), it was possible to visualise the fusion hybridisation signals on both der(7) and der(12), as this translocation is reciprocal. However, in one of the patients (no. 4), the translocation was accompanied by a deletion of the chromosome 7 region comprised between bands q22 and q36, in the same homolog affected by the translocation. A precise mapping of the deletion and trans-



location breakpoints enabled us to determine, in a previous study, that the chromosome 7 fragment translocated onto the der(12) included a small region from 7q22 joint with 7q36, indicating that the deletion event might have been prior to the translocation event [12]. In this case, only one fusion signal, on the der(12), was visible on both metaphase chromosomes and interphase nuclei, due to the fact that the chromosome 7 breakpoint occurred outside of the region covered by the probe. FISH images of metaphase chromosomes and interphase nuclei are shown in **Figure 2**. All of the samples used in this study had been previously analysed by FISH using various probe sets [2,10-12]. These are listed in **Table 1**. Two of the patients were previously studied using the three colour probe set (patient nos. 3 and 4) [10] and have been re-analysed here using this novel approach. The findings here reported confirmed analyses previously performed in most cases (patient nos 2-4) and refined the abnormality in one case (patient no. 1), where the chromosome 7 breakpoint was previously mapped between 7q35 and 7q36 and has now been narrowed down to between the regions flanking *HLXB9*. The dual colour FISH assay has the advantage over the three colour



one of relying on a fluorescence microscope equipped with the most commonly used filter sets. These recognise the red and green fluorochromes used to label the probes and the DAPI counterstaining. However, it has to be noted that the two derivatives cannot be distinguished based on the FISH pattern alone as they both carry a red-green fusion signal. Therefore, although the derivatives can be discriminated on the metaphases based on chromosome morphology, this is not possible when analysing interphase nuclei. Furthermore, a double fusion signal will be visualised only in cases of true reciprocal translocations without associated deletions. In the presence of deletion involving either of the chromosome 7 or 12 regions covered by the probes, or where one of the translocation breakpoints falls outside of the region covered by the probe, one fusion signal might be replaced by a single colour signal such as in case no. 4 here presented. In deletion cases, particular attention should be given to chromosome morphology as a large deletion might impact on the identification of the derivative. The dual colour FISH assay here presented has been designed to cover the regions flanking the *HLXB9* gene, to allow detection of the most common t(7;12) rearrangements. However, a certain heterogeneity in the chromosome 7 breakpoints has been described in the t(7;12) rearrangements [12], therefore situations in which one of the fusion signals is not visualised are likely to occur.

Conclusion

The availability of a dual colour FISH assay specific for the t(7;12)(q36;p13) will enable diagnostic laboratories to identify easily and confidently this cryptic chromosomal abnormality. The correct identification of this rearrangement will also help estimating its real incidence in infant leukaemia.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	TO	MV	CF	SS	ST
Research concept and design	--	✓	--	--	✓
Collection and/or assembly of data	✓	✓	--	--	✓
Data analysis and interpretation	✓	--	✓	✓	✓
Writing the article	--	--	--	--	✓
Critical revision of the article	--	--	✓	✓	✓
Final approval of article	✓	✓	✓	✓	✓
Statistical analysis	--	--	--	--	--

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