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Fluorescence in situ hybridization for del(5q) in myelodysplasia/acute myeloid leukemia: Comparison of *EGR1* vs. *CSF1R* probes and diagnostic yield over metaphase cytogenetics alone

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ABSTRACT

To determine the clinical utility of FISH for del(5q) in MDS/AML, we first compared FISH for 5q31 (*EGR1*) and 5q33 (*CSF1R*) in 51 myeloid neoplasms containing del(5q) by metaphase cytogenetics. Next, *EGR1* FISH was compared to metaphase cytogenetics alone in 269 cases of known or suspected MDS/AML. These studies show that while metaphase cytogenetics alone can detect del(5q) in most cases, FISH is particularly useful in cases with suboptimal growth. *EGR1* FISH detects del(5q) in a broad variety of myeloid neoplasms, including at least most cases of 5q− syndrome, while studies for *CSF1R* add little to the diagnostic yield.

1. Introduction

The identification of del(5q) assists in the diagnosis and classification of myelodysplastic syndromes (MDS) [1,2], and may also help to guide the choice of therapy, given the efficacy of lenalidomide in cases containing this abnormality [3,4]. FISH studies offer the potential to detect del(5q) much more rapidly than metaphase cytogenetic studies, which may have a turnaround time of several weeks, thereby facilitating clinical decision making. However, it is currently unclear which loci are the most appropriate to examine for detection of del(5q) in routine practice, and it is unclear in which situations FISH studies may increase the diagnostic yield over metaphase cytogenetics alone.

The breakpoints on chromosome 5q in MDS are heterogeneous, with the most common breakpoints identified by metaphase cytogenetic analysis consisting of 5q13 proximally and 5q33 distally [1,2]. Two minimally deleted regions have been described on chromosome 5q: the first region occurs in acute myeloid leukemia (AML) and high grade MDS and includes the early growth response 1 (*EGR1)* locus at 5q31 [5–7], while the second region, occurring in at least some cases reported as 5q− syndrome, is centered around the colony stimulating factor 1 receptor (*CSF1R*) locus at 5q33 [8–10].

The clinical utility of FISH for *EGR1* vs. *CSF1R* for the detection of del(5q) has not previously been reported. This study therefore examines whether FISH for *EGR1*, *CSF1R* or a combination of both loci provides the greatest sensitivity for detection of del(5q) in 51 cases of MDS/AML containing this abnormality by metaphase cytogenetics. Next, we also examine the additional diagnostic yield of FISH studies over metaphase cytogenetics alone for detection of del(5q) in 269 cases of known or suspected MDS and AML.

2. Materials and methods

2.1. Case selection

All studies were approved by the Cleveland Clinic Institutional Review Board. First, a search of the Cleveland Clinic cytogenetics database from 2001 to 2006 identified 51 cases of myeloid neoplasms with available archived material for FISH analysis and del(5q) previously demonstrated by metaphase cytogenetic studies. Next, a search of the cytogenetics database from January 2007 to December 2008 identified 269 bone marrow samples obtained for known or clinically suspected MDS and AML which were submitted to the Cleveland Clinic Cytogenetic Laboratory for metaphase cytogenetic studies and FISH for *EGR1* deletion.

2.2. Metaphase cytogenetics

Metaphase cytogenetic studies were performed in the Cleveland Clinic Cytogenetics Laboratory according to standard methods. Chromosome preparations were G-banded using trypsin and Giemsa (GTG) and karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) [11].

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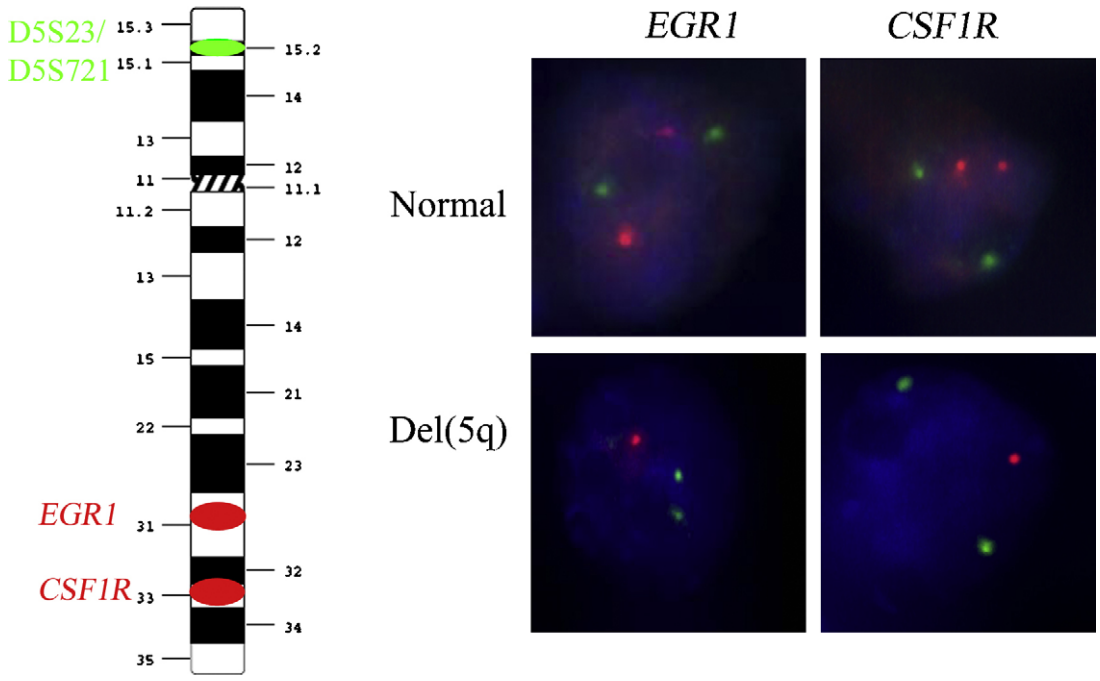

Fig. 1. FISH probes for del(5q) include a reference probe labeled with Spectrum Green (*D5S721*,*D5S23*) and a probe for either *EGR1* or *CSF1R* labeled in Spectrum Orange. Positions of each probe on chromosome 5 are illustrated on the accompanying ideogram. A normal nucleus exhibits two red and two green signals, while a nucleus with loss of the *EGR1* or *CSF1R* locus display two green and one red signal. Ideogram reproduced with permission from Idiogram Album: Human, copyright 1991 David Adler and Michael Willis.

2.3. FISH analysis

In the first patient cohort (51 patients with known del(5q)), FISH studies were performed on bone marrow aspirate coverslips ($n = 45$), cytogenetics cell pellets ($n = 4$), or formalin fixed, paraffin embedded bone marrow clot sections ($n = 2$) using dual color probes for *EGR1*/*D5S721*,*D5S23* and *CSF1R*/*D5S721*,*D5S23* (Abbot Molecular, Abbott Park, IL) as previously described [12]. Briefly, aspirate coverslips or cell pellets were fixed in Carnoy's solution, washed, and subjected to proteinase K treatment. Following wash treatment, 10 μL probe solution was applied and probe and target DNA allowed to codenature at 73 °C for 5 min and hybridize overnight at 37 °C. Slides were counterstained with DAPI and signals were visualized on an Axioskop photomicroscope (Zeiss, Oberkochen, Germany). For bone marrow clot sections, 5 μm paraffin sections were baked overnight at 60 °C, deparaffinized, subjected to proteinase K treatment and treated as above. Nuclei displaying two green (*D5S721*,*D5S23* reference probe) and two red (*EGR1* or *CSF1R*) signals were scored as normal, and nuclei displaying two green and one red signal were scored as abnormal. At least 100 cells were counted in most cases. In cases with limited cellularity, fewer than 100 countable cells were accepted if >25 cells were abnormal. Thresholds for interpretation as a positive result were established by evaluation of 20 normal bone marrow preparations (for whole cell samples) and 10 formalin fixed paraffin embedded tonsils (for paraffin embedded clot section samples). Cutoffs were established at the mean plus 3 SD as follows: >6% for *EGR1* or *CSF1R* in whole cell preparations and >10% for *EGR1* or *CSF1R* probes in paraffin sections. In the second cohort (269 bone marrows with known or suspected MDS/AML), FISH for *EGR1*/*D5S721*,*D5S23* (Abbott) was performed on cytogenetic cell pellets at the Cleveland Clinic ($n = 80$) as described above or at an outside reference laboratory ($n = 189$). Both laboratories employed a similar cutoff for interpretation as an abnormal result.

3. Results

To compare the utility of *EGR1* and *CSF1R* probes for detection of del(5q), FISH studies for *EGR1* and *CSF1R* deletion were performed in 51 cases of myeloid neoplasms with del(5q) previously identified by metaphase cytogenetic studies (Fig. 1 and Table 1). Two cases were analyzed by *EGR1* FISH only due to insufficient archived material for *CSF1R* FISH studies. Overall, *EGR1* deletion was detected in 49/51 (96%) and *CSF1R* deletion was detected in 45/49 (92%) of cases analyzed ($p = 0.43$). Cases with negative FISH results with at least one probe are detailed in Table 2. Two cases displayed deletion of *EGR1* but were negative for *CSF1R* deletion: one MDS/MPD overlap syndrome and one AML. Two cases, one MDS/MPD overlap syndrome and one AML, each displayed del(5q) by metaphase cytogenetics but were negative for *EGR1* and *CSF1R* deletion by FISH.

Next, we evaluated the utility of *EGR1* FISH for detection of del(5q) and metaphase cytogenetics alone in 269 bone marrows obtained for known or clinically suspected myelodysplasia or acute myeloid leukemia (Table 3). Metaphase cytogenetic studies demonstrated abnormal karyotypes in 78 cases, including loss of chromosome 5q in 17 cases. In each case with del(5q) identified by metaphase cytogenetics, FISH also demonstrated loss of *EGR1* (17/17, 100%). Each of the 61 cases with abnormal

Table 1
Comparison of FISH for *EGR1* vs. *CSF1R* in 51 myeloid neoplasms containing del(5q).

Diagnosis	*EGR1* FISH positive	*CSF1R* FISH positive
5q− syndrome	8/8 (100%)	6/6 (100%)
Refractory cytopenia with multilineage dysplasia	6/6 (100%)	6/6 (100%)
Refractory anemia with excess blasts	8/8 (100%)	8/8 (100%)
MDS, unclassifiable	1/1 (100%)	1/1 (100%)
MDS, therapy-related	1/1 (100%)	1/1 (100%)
MDS/MPD overlap syndrome, unclassifiable	5/6 (83%)	4/6 (67%)
Acute myeloid leukemia	20/21 (95%)	19/21 (90%)
Total	49/51 (96%)	45/49 (92%)