Available online at www.sciencedirect.com
ScienceDirect
Leukemia Research 31 (2007) 1185–1189
Leukemia Research
www.elsevier.com/locate/leukres

Efficacy of conventional cytogenetics and FISH for EGR1 to detect deletion 5q in hematological disorders and to assess response to treatment with Lenalidomide

Ying S. Zou [a], Stephanie R. Fink [a], Kimberly J. Stockero [a], Sarah F. Paternoster [a], Stephanie A. Smoley [a], Han W. Tun [b], Craig B. Reeder [c], Ayalew Tefferi [a], Gordon W. Dewald [a,*]

[a] *Cytogenetics and Hematology, Mayo Clinic, Rochester, MN, United States*
[b] *Hematology, Mayo Clinic, Jacksonville, FL, United States*
[c] *Hematology, Mayo Clinic, Scottsdale, AZ, United States*

Received 30 August 2006; received in revised form 11 October 2006; accepted 12 October 2006
Available online 27 November 2006

Abstract

In clinical practice, whether FISH for EGR1 in interphase nuclei has similar efficacy to detect deletion 5q anomalies as conventional cytogenetic studies is unknown. We compared conventional cytogenetics and FISH for 145 patients with deletion 5q and detected this anomaly by both methods in 144. Nine patients with myelodysplasia were studied before and after treatment with Lenalidomide and results were concordant for 28 of 29 specimens. FISH did not detect anomalies other than deletion 5q in 31 patients. This study suggests FISH is useful to detect deletion 5q, but is not a substitute for conventional cytogenetics.

Keywords: Deletion 5q; EGR1; Myelodysplasia; Acute leukemia

1. Introduction

Recently, List et al. [1–3] reported that patients with myelodysplasia who have an abnormal clone with deletion 5q in their bone marrow are particularly responsive to treatment with Lenalidomide. This work was confirmed in a large international clinical trial by Celgene Corporation [3]. In 2006, Lenalidomide received FDA approval for myelodysplasia associated with deletion 5q based on conventional cytogenetic results before and after treatment.

A good deal has been published about deletion 5q in myelodysplasia and acute myeloid leukemia, but the precise gene or genes involved in this chromosome anomaly are not yet known [4,5]. Some patients are characterized by the 5q syndrome, but most have various forms of myelodysplasia; some of these patients evolve to acute myeloid leukemia [6]. Some patients have deletion 5q as the only abnormality while others have one or more subclones with deletion 5q and additional chromosome anomalies [7,8]. These additional chromosome anomalies arise by chromosome evolution and are strongly associated with survival [7,9,11].

The deletion 5q is an interstitial deletion involving the q-arm. Breakpoints are usually at 5q13 and 5q33, but variations in breakpoints result in observation of a wide variety of different forms of deletion 5q in clinical practice. Most deletion 5q clones lack a small segment of chromosome 5 in 5q31.1 [12–14]. At least 28 genes are known to occur in this critical deletion region, though none have been specifically associated with the pathogenesis of the deletion 5q anomaly [4]. Some investigators believe that deletion 5q is a two-step process. One process involves loss of critical genes by way of the

* Corresponding author at: Division of Laboratory Genetics, Mayo Clinic, 200 First St. SW, Rochester, MN 55905, United States. Tel.: +1 507 284 7278; fax: +1 507 284 0043.
E-mail address: gdewald@mayo.edu (G.W. Dewald).

0145-2126/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2006.10.011

deletion 5q while the other process may involve a mutation of one or more genes on the normal chromosome 5 homolog [15]. This hypothesis has not yet been confirmed by experimental procedures.

In clinical practice, the deletion 5q can be detected by conventional cytogenetic methods by analyzing mitotic cells in bone marrow. In recent years, a fluorescent labeled probe for early growth response gene 1 (EGR1) at 5q31.2 has been used to observe deletion 5q in non-proliferating interphase nuclei in both bone marrow and peripheral blood [16]. Clinicians often inquire about the best cytogenetic or molecular cytogenetic methods to workup patients with deletion 5q. In clinical practice, FISH studies of interphase nuclei with probes for EGR1 are widely used to detect deletion 5q, but it is not known whether this method has similar efficacy as conventional cytogenetic studies. Thus, we compared results for conventional cytogenetic and FISH studies on the same specimens for 145 patients with various forms of deletion 5q.

2. Methods

This study was performed with approval of the Mayo Foundation Institutional Review Board and informed consent was provided according to the Declaration of Helsinki. We reviewed our cytogenetic database for patients with various forms of deletion 5q in similar proportions to patients observed in our clinical practice [7]. We identified 145 patients in our database that met these criteria. This series included nine patients with myelodysplasia that were studied before and after treatment with Lenalidomide.

This cohort consisted of 109 females and 36 males who ranged in age from 40 to 96 years of age. The hematological disorder for each patient was classified into myelodysplasia, myeloproliferative, acute myeloid leukemia or lymphoproliferative disorder based on clinical information provided by the referral physician at the time the specimen was submitted for chromosome studies.

For each patient, conventional cytogenetic studies were done on up to 20 metaphases from bone marrow specimens that were processed by standard methods for 24- and 48-h unstimulated cultures with Chang medium BMC® [17]. Interphase FISH studies were done on the leftover bone marrow specimens that were used for conventional chromosome studies. FISH was done on 200 interphase nuclei for a set of FISH probes designed to detect anomalies of chromosomes 5 as previously described [18]. FISH studies were done with commercial probes (Vysis Inc., Downers Grove, Illinois) for EGR1 (5q31.2) and D5S23/D5S721 (5p15.2). The normal cut-off for deletion 5q with this FISH method was <6.0% based on 200 consecutive scoreable nuclei.

3. Results

Among the 145 patients in this investigation, 104 were referred for myelodysplasia, 9 for acute myeloid leukemia, 9 for lymphoproliferative disorders, 7 for myeloproliferative disorders, and 16 without clinical information.

A total of 116 patients had only deletion 5q, 22 had deletion 5q, and one other anomaly and 7 had deletion 5q plus two or more anomalies. Breakpoints of the various deletion 5q chromosomes observed in this study are summarized in Fig. 1 along with representative G-banded images for these anomalies. A total of 116 patients had del(5)(q13q33), 17 del(5)(q15q33), 3 del(5)(q22q33), 1 del(5)(q11.2q33), 1 del(5)(q12q33), 1 del(5)(q13q22), 1 del(5)(q13q31), 1 del(5)(q13q35), 1 del(5)(q15q31), 1 del(5)(q22q35), 1 del(5)(q31q33), and 1 del(5)(q31q35) (Fig. 1). Deletion 5q was detected in 144 patients by both conventional cytogenetics and FISH. One patient had a del(5)(q13q22) in 20 of 20 metaphases but was normal by FISH.

The mean percentage of deletion 5q cells was 85 ± 18.9 (range 20–100) by conventional cytogenetics and 62.4 ± 21.5 (range 2–99) by FISH. The percentage of abnormal cells by FISH was lower in 135 of 145 patients compared to

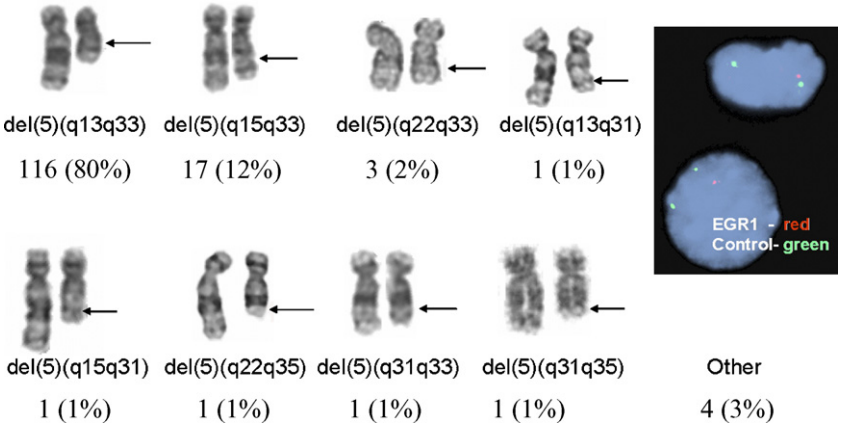

Fig. 1. Representative deletion 5q anomalies and proportion of patients with these anomalies in this investigation. Representative FISH image of two interphase nuclei showing deletion of an EGR1 signal associated with deletion 5q.