

## Chromosome 7 monosomy and deletions in myeloproliferative diseases

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### Abstract

We studied deletion and monosomy of chromosome 7 in 150 patients with myeloproliferative diseases. We found 8/150 patients with monosomy 7 by cytogenetics and 4/150 with deletions of the long arm of chromosome 7 by restriction fragment length polymorphism (RFLP) analysis performed with Southern and polymerase chain reaction. To overcome limitation of RFLP analysis, we restricted loss of heterozygosity study with microsatellites to 45 patients, observing deletion 7q31.1 in 7/45 patients. In all patients with molecular alterations the deletion was observed only in myeloid cells, while the monosomy was detected in both myeloid precursor and lymphocytes. This finding suggests a CD34-totipotent stem cell origin for the monosomy and a colony forming unit – granulocyte, erythrocyte, monocyte, megakaryocytes (CFU–GEMM) stem cell origin for the deletions.

**Author Keywords:** Myeloid leukemia; Myelodysplasia; DNA; Deletion; Chromosome 7; Microsatellite; Cytogenetics

**Abbreviations:** LOH, loss of heterozygosity; t-AML, therapy related acute myeloid leukemia; t-MDS, therapy related myelodysplastic syndromes; PCR, polymerase chain reaction; CML, chronic myeloid leukemia; ET, essential thrombocytemia; PV, polycytemia vera; RFLP, restriction fragment length polymorphism; MF, myelofibrosis; TSG, tumor suppressor gene; CFU–GEMM, colony forming unit – granulocyte, erythrocyte, monocyte, megakaryocytes

## 1. Introduction

Monosomy of chromosome 7 or partial deletion of the long arm of chromosome 7 are often observed in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) after exposure to mutagenic agents or radiation therapy [1] and are more frequently present in therapy related myelodysplastic syndromes (t-MDS) and therapy related acute myeloid leukemia (t-AML) [2].

Molecular analysis of the 7q deletions, in myeloid leukemias, has recently shown that at least three regions of the long arm of chromosome 7 are involved: 7q22, 7q31.1, and 7q36 [3].

A recessive mechanism of tumorigenesis has been suggested for these as for many other deletions [4] and these chromosomal abnormalities have been associated with a rapid progression of the disease, and a poor response to therapy [5].

The cell origin of MDS and AML could be either the CFU–GEMM stem cell or the CD34-totipotent stem cell, but neither of these possibilities has yet been proved [6].

In our patients, we made a careful determination of the presence or the absence of abnormalities in stem cells, committed cells and derivative cells.

Indeed myeloid cells were separated from lymphocytes with antibodies conjugated to magnetic beads to obtain a pure fraction that we considered as tumoral cells while lymphocytes were considered the normal counterpart.

In this report, we studied 150 patients affected by myeloproliferative diseases to search for monosomy and deletions of chromosome 7 by cytogenetics, Southern blot and polymerase chain reaction (PCR) of restriction fragment length polymorphism (RFLP).

The finding of a small number of deletions, 4/150 (2.7%) is due to the need of large amount of DNA and to a low informativeness of RFLP markers (whose heterozygosity is lower than microsatellites).

These limitations led us to study loss of heterozygosity (LOH) with microsatellite markers using a fluorescent PCR system on 45 patients with MDS, AML, and t-AML since microsatellites are highly informative [7]. Moreover microsatellites will also be useful to narrow the region, where to search for a putative tumor suppressor gene (TSG).

## **2. Patients materials and methods**

### **2.1. Patients**

One hundred and fifty patients with myeloproliferative diseases were studied for chromosome 7 alterations. 24 patients were secondary AML, 43 MDS, 31 de novo AML, 29 chronic myeloid leukemia, 7 essential thrombocytemia (ET), 12 polycytemia vera (PV) and 4 myelofibrosis (MF).

### **2.2. Cytogenetics**

Chromosome analysis on all 150 patients was performed on the bone marrow of the patients, without any stimulation, and this has been considered as the karyotype of the myeloid cells. Cytogenetics analysis of the cultured

peripheral blood cells stimulated by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for few days as described [8], was carried out only to study the karyotypes of the lymphocytes. Chromosomes were G-banded with Wright stain or R-banded with acridine orange. Karyotypes were described according to ISCN nomenclature [9].

### **2.3. Cell separation**

Peripheral blood cells from these patients were separated into lymphocytes and myeloid cells either by ficoll gradient or by mini macs columns and monoclonal antibodies for the myeloid antigens CD15 and CD33 on magnetic beads.

Fractions were smeared and Giemsa colored for microscopical examination to detect cell separation, more than 95% myelomonocytic cells were requested to include these samples as tumor cells, normal cells were mostly lymphocytes.

### **2.4. DNA**

DNA was extracted from the two fractions by Hirt buffer, proteinase K, phenol–chloroform extraction and ethanol precipitation as described [10].

PCR for RFLP studies was performed as described in Ref. [11] with 30 cycles at 93°C for 1 min, 55°C for 1 min and 72° for 1 min.

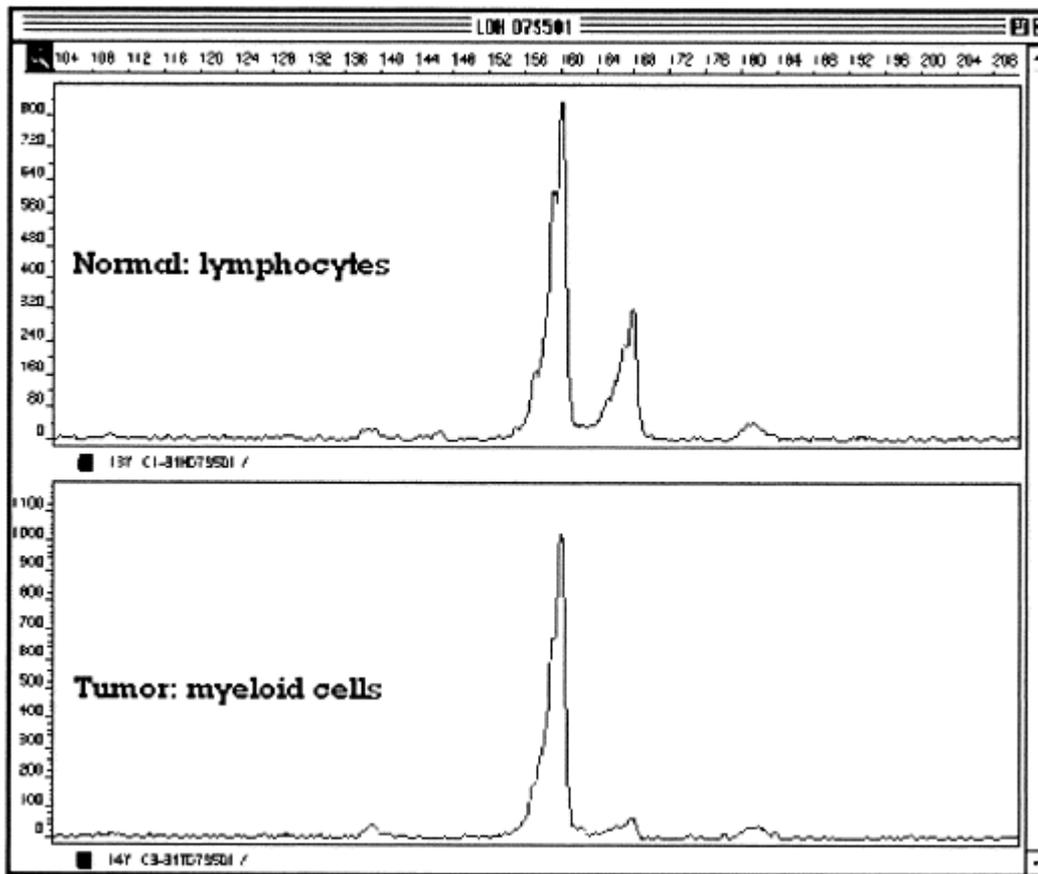
### **2.5. Microsatellites analysis**

Microsatellites analysis was made on 45 patients with MDS, AML, t-AML with 12 microsatellites mapped in 7q22-7q31.1. One primer of each pair was labeled with a fluorescent marker (hex, 6-fam or tet) to obtain a fluorescent product of amplification.

PCR was performed in a 50 µl mixture containing 10 pmol of each primer, 200 µM dNTPs, 1.5 mM MgCl<sub>2</sub>, 1.25 U TaqGold polymerase (Perkin Elmer, Foster City, CA), 1×PCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl) and 20 ng of DNA template. Thirty-nine cycles of denaturation at 94°C for 1 min, annealing 55°C for 1 min, and extension 72°C for 1 min were carried out.

Three microliters of fluorescent PCR product were run on the fluorescent Capillary System ABI Prism 310 (Applied Biosystem, Foster City, CA), results were evaluated using the Gene Scan Software.

LOH was assessed comparing the difference in the height of allele peaks between normal and tumor samples according to mathematic model described by Canzian et al. [12] and an example is shown in [Fig. 1](#).



**Fig. 1. Example of microsatellite analysis (D7S501). Different CA repeats of the two alleles are shown as two peaks with different molecular weights (horizontal scale). LOH is shown by the loss of the second allele in the tumor sample.**

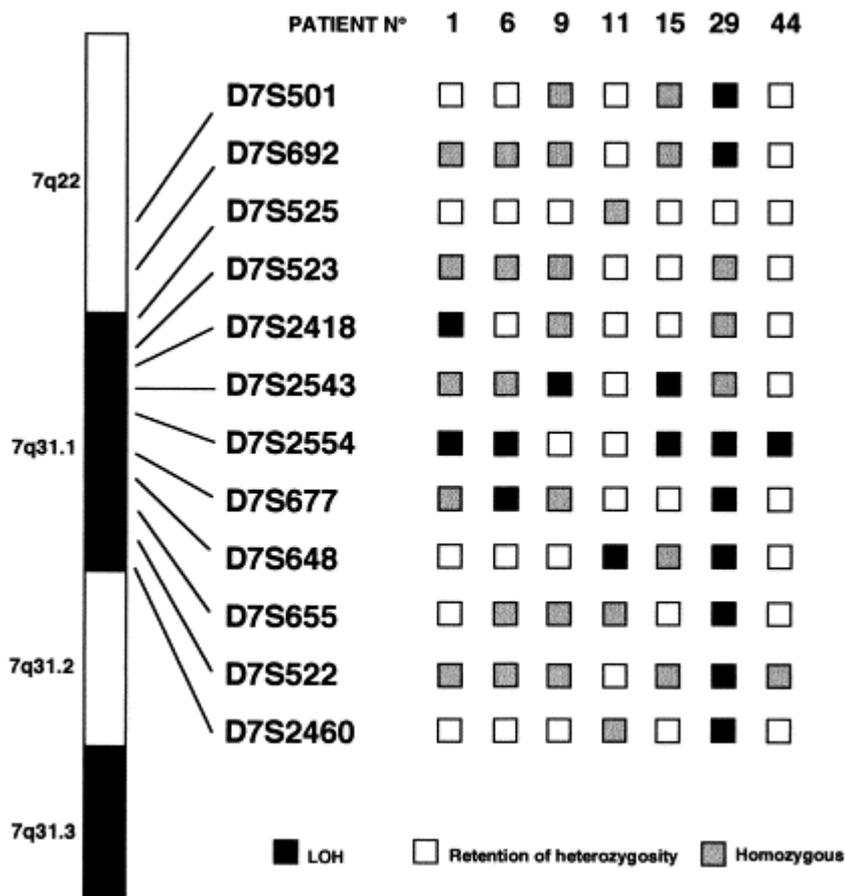
### 3. Results

We identified by cytogenetics, monosomy of chromosome 7 in 8/150 patients (3 t-AML, 2 AML, and 3 MDS); no deletions of chromosome 7 were found.

Southern blot analysis on all patients was made with met oncogene (7q31.1) as probe and we found 3/150 patients with deletion in this locus (2 t-AML and 1 MDS). Other probes used, were collagen gene COL1A2, multidrug resistance gene, erythropoietin gene and elastin gene. No deletions were found with these probes, all of them are centromeric to the met locus.

PCR with RFLP study identified only 1/150 patients with LOH in 7q22 (t-AML).

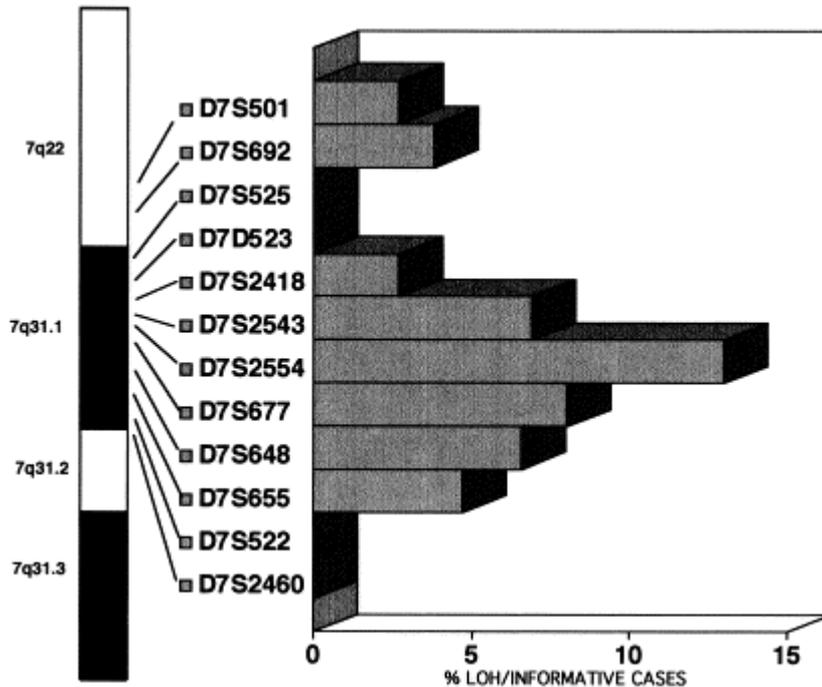
Microsatellite analysis was carried out on 45 patients and LOH was observed in 7/45 patients ([Fig. 2](#)). These patients were affected by: 3 t-AML, 2 AML, and 2 MDS.



**Fig. 2. Summary of allelic losses mapped on 7q22–31.1 in myeloproliferative diseases. Only cases with LOH in at least one locus are illustrated.**

The most deleted region is in 7q31.1 around D7S2554, found in 5/7 patients. All deletions seem to be interstitial since all patients retained the more telomeric region 7q35–36 studied with a RFLP of the beta chain T-cell receptor gene [13].

In only 1/7 patients the deletion was found also in 7q22 (since only 2 microsatellites are located in this region) as shown in [Fig. 3](#).



**Fig. 3. Graphic representation of human chromosome 7q22–31.3 and approximate position of microsatellite repeats. The histogram shows the percent of LOH over informative cases, studied with microsatellite analysis. The most deleted region is between D7S2418 and D7S655 with loss of microsatellite D72554 in 5 out of 38 informative patients (13%).**

#### **4. Discussion**

The results obtained suggest some observations on the cellular origin of chromosome 7 monosomy and deletions. The absence of deletions in peripheral lymphocytes (derived from the totipotent stem cell as the myeloid precursor CFU–GEMM) argues against a CD34-totipotent stem cell origin of the deletion and suggests a CFU–GEMM cell origin.

Cytogenetics studies made on lymphocytes of the patients with monosomy 7 seem to show, on the other side, that the deletion starts in a CD34-totipotent stem cell, since the monosomy is observed both in lymphocytes and CFU–GEMM derived myeloid cells.

The studies of LOH in tumors are made to identify putative TSGs. Many studies have found a higher rate of LOH than of TSGs. This may be due to many problems in the identification of candidate TSGs, because they may be inactivated by different mechanisms, including small deletions, point mutation, homozygous deletion and hypermethylation of promoter sites [[14](#)].

The clinical influence in patients with LOH and monosomy of different chromosomes is not yet completely clear, as discussed in Refs. [[15](#) and [16](#)].

In our study the clinical course of patients with chromosome 7 alterations has been, in all patients, very aggressive with a poor response to the therapy and a short survival.

We identified by cytogenetics monosomy of chromosome 7 in 8/150 patients (3 t-AML, 2 AML and 3 MDS); 5/8 patients with monosomy were affected by AML and t-AML at the time of cytogenetics finding.

The other three patients developed a very aggressive AML after a latency from the diagnosis of MDS, of 3, 8 and 14 months. All three patients had a very short survival after the diagnosis of AML.

Considering also the 11 patients with deletions (7q22, 7q31, and 7q31.1) our results showed that 19/150 (12.6%) patients had alterations of chromosome 7 (monosomy and deletions).

The most deleted region was identified around the genetic locus D7S2554, this LOH was found in 5/7 patients with deletions detected by microsatellites analysis.

Also these patients had a very poor clinical course but one patient with MDS, alive after 8 yr, showed deletions in D7S2418, D7S2554 and in the met locus (Southern blot, data not shown).

Further studies with newly identified markers will help to search for differences in the deletion of this patient and will narrow the minimal region of loss in MDS, AML, and t-AML.

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