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Structural analysis and expression profile of a novel gene on chromosome 5q23 encoding a Golgi-associated protein with six splice variants, and involved within the 5q deletion of a Ph(−) CML patient

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Abstract

We have identified a novel gene, upstream of the cytokine gene cluster region in 5q23–31, residing within one of the most common deleted segments associated with MDS. The novel gene exhibits significant alternative splicing generating at least six splice variants encoding four putative proline-rich protein isoforms, one of which is Golgi-associated. The gene is ubiquitously expressed and conserved among species with the *C. elegans* homologue being the most interesting, since it resides within an operon with two other genes, phospholipase D and dishevelled, a member of the Wnt pathway, suggesting a functional association. In addition, the novel gene and other key regulatory genes of the region, such IL3, Ril, AF5q31 and TCF-1, were found to be deleted in an atypical CML case, thus underscoring the significance of this subregion in the leukemogenesis process.
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Keywords: 5q23–31; Centromeric to the IL3-TCF-1 subregion; Splice variants; Proline-rich N-terminus; Lipase motif; Potential lipid-modifying enzyme; Wnt signaling pathway

1. Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of hematopoiesis, characterized by cytopenias, ineffective hematopoiesis and hypercellular bone marrow [1,2]. Consistent loss of genetic material of the long arm of chromosome 5 in a subset of myeloid disorders such as the 5q− syndrome [3] has led to the speculation that deletion of one or more regulatory genes, including tumor suppressor genes, may play an important role in the generation of the phenotype of MDS and its evolution to leukemia [4]. The pathogenesis of the syndrome and its leukemogenic potential may share similar but more complex mechanisms to those of retinoblastoma. Specifically, it seems that the eventual leukemic transformation of the myeloid precursor cell, involves a series of multistep processes and pathways affecting the deregulation of multiple genes [5]. Furthermore, it is conceivable that multiple genetic pathways can be activated generating several distinct clones in the same patient [5–7].

The region of chromosome 5 deleted in MDS contains an impressive number of hematopoietic growth factors and

Abbreviations: MDS, myelodysplastic syndromes; IL3, interleukin 3; TCF-1, T-cell transcription factor 1; GM-CSF, granulocyte macrophage-colony stimulating factor; IRF1, interferon regulatory factor 1; cDNA, complementary deoxuribonucleic acid; YAC, yeast artificial chromosome; PAC, P1 artificial chromosome; Wnt proteins, secreted signal molecules controlling development; FISH, fluorescence in situ hybridisation; Ph(−), Philadelphia chromosome negative; CML, chronic myeloid leukemia; NIX, nucleotide identification X; PCR, polymerase chain reaction; BAC, bacterial artificial chromosome; MTC, multiple tissue panel; RACE, rapid amplification of cDNA ends; UTR, untranslated region; RNA, ribonucleic acid; GFP, green fluorescent protein; SH3, src homology domain 3; DUF, domain of unknown function; N-terminus, amino-terminus; C-terminus, carboxy-terminus

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receptors [4] but exhibits a significant cytogenetic heterogeneity which has led to the identification of different putative "critical" subregions on 5q, based on the individual patient series [8–11]. Our group has focused on the 5q23 region encompassing the IL3/GM-CSF and TCF-1 genes. The importance of this subregion in its involvement in MDS and leukemogenesis, has been prompted by an informative patient with MDS undergoing transformation to acute leukemia and exhibiting an acquired deletion of one GM-CSF allele and a rearrangement of the other allele in bone marrow DNA [12]. Several studies on 5q region have revealed a number of tumour suppressor and putative regulatory genes such as IRF1 [13], Ril [14], GM-CSF and IL3 [15], located within the deleted segments, which have been considered as candidates for contributing to the syndrome. However, none of these genes has been convincingly proven to participate either in the generation of the MDS or in the early events of leukemogenesis [16–23]. Therefore, more comprehensive approaches are needed to delineate the structural and functional properties of these candidate subregions and their involvement in leukemogenesis. To this end, our group has initiated a systematic analysis of this subregion by generating a physical and transcript map employing a series of molecular and bioinformatics approaches, such as cDNA selection techniques [24] with appropriate cDNA libraries and analysis of YAC and PAC genomic clones [25–29].

In the present study, following these approaches, we identified and characterized one of the novel genes isolated from this region, along with its six splice variants encoding four putative protein isoforms, which may be involved in the Wnt signaling pathway. We also show that the novel gene is ubiquitously expressed and one of its protein isoforms is associated with the Golgi apparatus. In addition, by a combination of metaphase and interphase FISH we have unambiguously established for the first time both the order of a collection of genomic clones (including the novel gene and known genes such as IL3, Ril, AF5q31 and TCF-1), and the definitive orientation of the region within the context of the long arm of chromosome 5, i.e. cen-AC005611-AC008426-IL3/GM-CSF-RIL-AF5q31-TCF-1-AC006077-tel. Finally, to further assess the significance of this region and its involvement in clonal myeloid disorders, we have performed a systematic FISH analysis for the presence of the subregion in a case of a Ph(−) chronic myelogenous leukemia patient with a complex translocation between the two homologues of chromosome 5, as the sole aberration, leading to a deletion of 5q21 to 5q33 [30]. The critical subregion containing the novel gene, IL3, Ril, AF5q31 and TCF-1 genes was found to be deleted in this atypical CML, underscoring the significance of this subregion in the leukemogenesis process.

2. Materials and methods

2.1. NIX analysis and verification of predicted exons by PCR analysis of a cDNA library panel

Potential exons in BAC clone AC005611 were identified by using the NIX platform (http://menu.hgmp.mrc.ac.uk/menubin/Nix/Nix.pl). Nine primers (26F, 33F, 47F, 60F, 60R, 62R, CDSF, 60698R, AJvarR) as shown in Table 1, derived from these exons were used for expression studies

Table 1
List of primer sequences

	Primer name	Primer sequence	Nucleotide position in genomic clone AC005611
1	26F	5′-taacttccagggtgcgccttcgtt-3′	26235–26258
2	26R	5′-aaagtgcctgcgtggtcgctgaa-3′	26333–26311
3	32F	5′-gaagaaaaatgatggaagagagtgg-3′	32037–32061
4	32R	5′-gaacaggggtagaagacatagcagtagc-3′	32141–32114
5	CDSF	5′-atgatggaagagagtggaatagagacaaca-3′	32045–32074
6	33F	5′-cggcaaccagttctttttcttctcca-3′	33096–33121
7	33R	5′-ccgaaggcagcagcagttgatgga-3′	33300–33277
8	33Rnew	5′-tcctgaaggtgcagaaaatgatggc-3′	33481–33457
9	35F	5′-ttttgccaactcctattactcagcaagcc-3′	35186–35214
10	42F	5′-tggaggtgaactggatattgtagtgacc-3′	42188–42215
11	4R	5′-actggttgtggggcaatattggactg-3′	42325–42300
12	47F	5′-tcaggaacggatagatagcttgcgtc-3′	47614–47639
13	60F	5′-gaggtggtcaggccttttggtgac-3′	60297–60320
14	60Fnew	5′-ctgggatgtcccgtcggcagatg-3′	60386–60408
15	60R	5′-tcctgggtggcaggcgctgttta-3′	60466–60444
16	60698R	5′-gatttgtttctttttgtctcctggaatg-3′	60660–60633
17	NF	5′-cctacctgggagactgagactttcc-3′	60482–60506
18	NR	5′-gtctctgcacatgaatttccaccttt-3′	60777–60753
19	62F	5′-atccccaaagagcagtggcagtc-3′	62372–62394
20	62Fn	5′-agaaattttcctgcccctggtgacct-3′	62413–62438
21	AjvarR	5′-atcttcctcacccactcacacggtt-3′	62481–62457
22	Ajv12R	5′-gaacatagaatattatcaatttggcttccc-3′	63484–63455

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