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High level expression of *N*-acetylgluosamine-6-*O*-sulfotransferase is characteristic of a subgroup of paediatric precursor-B acute lymphoblastic leukaemia

Georgina Timson [a], Sri Banavali [b], Marina I. Gutierrez [a], Iain Magrath [c], Kishor G. Bhatia [a], Malcolm H. Goyns [a,*]

[a] *Research Centre at KFNCCCR, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia*
[b] *Tata Memorial Hospital, Mumbai, India*
[c] *INCTR, Brussels, Belgium*

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Abstract

Microarray analysis is a powerful technology, but its impact on routine diagnosis for the near future maybe in revealing individual genes, which are useful diagnostic markers. Recently microarray analysis has identified a novel subgroup of childhood precursor-B acute lymphoblastic leukaemia (ALL) from a unique gene expression profile of over 30 genes. We have evaluated the four most highly expressed genes from this profile, by quantitative real time RT-PCR, to determine whether any of these genes by itself could be useful as a diagnostic indicator. The levels of expression of *N*-acetylglucosamine-6-*O*-sulfotransferase (GN6ST), protein tyrosine phosphatase receptor M (PTPRμ), G protein-coupled receptor 49 (HG38) and KIAA1099 protein were determined in childhood precursor-B ALL samples from a cohort of 116 Indian patients. In nine cases, three or four of these genes exhibited very high expression levels, but only GN6ST was consistently over-expressed. We suggest that very high level expression of GN6ST is a useful diagnostic marker for a subgroup of previously unclassified ALL.
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1. Introduction

Paediatric ALLs are a heterogeneous group of diseases, which by using risk-adapted therapy that involves the tailoring of the intensity of treatment to each patient's risk of relapse, have achieved significant long-term survival rates [1]. Critical to this approach has been the accurate assignment of individual patients to specific risk subgroups. Cytogenetic analysis has led to the identification of five subgroups in precursor-B ALL. These are characterized by the presence of rearrangements of BCR-ABL due to t(9;22), E2A-PBX1 due to t(1;19), TEL-AML1 due to t(12;21) and MLL gene due to alterations of chromosome 11q23. In addition, the presence of a hyperdiploid karyotype (greater than 50 chromosomes) has been recognized as a distinct subgroup [2]. It appears that the different genetic lesions underlying these different subtypes influence the response of the patient to cytotoxic drugs. For example, the E2A-PBX1 subgroup responds poorly to conventional antimetabolite-based treatment, but has

* Corresponding author. Address: School of Health, Nursing & Midwifery, University of Paisley, Paisley, PA1 2BE, Scotland, UK. Tel.: +44 141 8494284; fax: +44 141 8494203.
E-mail address: malcolm.goyns@paisley.ac.uk (M.H. Goyns).

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cure rates approaching 80% when treated with more intensive therapies [3].

One of the problems with the above molecular characterizations is that the majority of ALL cases still remain as 'unclassified'. It is therefore of considerable interest to identify markers, which are of value in defining cases within this unclassified group. The recent introduction of DNA microarrays, which enable gene expression profiles to be identified, has provided a powerful new approach to the classification of cancers [4,5], including ALL [6–8]. These studies have indicated that it is now possible to use a single technology platform to obtain a genome-wide approach to the classification of ALL. This has also enabled the accurate prediction of certain patients who are at high risk of failing therapy. As technical developments occur, for example through enhancing the representation of gene sequences present on commercially available microarrays, so it is likely that the predictive power of this technology will improve further.

Microarray technology is, of course, expensive and technically demanding and is therefore unlikely to become a routine diagnostic procedure in most health centres in the near future, particularly in developing countries. However, by using this technology in a research programme it may be possible to identify key genes of diagnostic importance, which can then be studied using simpler assay systems that would be of utility in routine diagnosis. Recently, a major study of paediatric ALL using microarray technology has revealed that a novel subgroup, defined by the expression profile of over 30 genes, can be identified among unclassified cases [8]. We have therefore examined the four most highly expressed genes in this subgroup's gene expression profile to determine whether any of them could be used individually as a reliable diagnostic marker for a subgroup of hitherto unclassified ALL.

2. Materials and methods

2.1. Patient samples

Samples were collected from children with precursor-B cell ALL. The children had a median age of 5 years (range, 0–14 years). Bone marrow aspirates or peripheral blood samples were collected at the time of routine diagnostic procedures. Written informed consent was obtained from the guardians under approved Institutional Review Board projects. We examined a random selection of 91 patient samples with unclassified ALL. In addition, a number of samples were selected from defined subgroups. The latter were TEL/AML (nine samples), BCR/ABL (eight samples), E2A/PBX (six samples) and MLL (two samples). We also included three T-ALL samples, two normal bone marrow and two normal peripheral blood samples for comparison. Most of the samples investigated were bone marrows, but 21 were peripheral blood leukocyte samples.

2.2. Cell lines

The RS4;11 (pre-B), Yt (NK), jurkat, Reh and K562 cell lines were cultured in RPMI 1640 with 10% FCS in a humidified incubator with 5% carbon dioxide at 37 °C.

2.3. RNA extraction and cDNA preparation

Patients' mononuclear cells were obtained from ficoll-hypaque density gradient centrifugation and lyzed with TRIZOL reagent (Invitrogen Life Technologies) for RNA extraction. The quality of the extracted RNA was checked by absorbance at 260 and 280 nm and gel electrophoresis. To prepare cDNA, 2.5 μg total RNA from either patient samples or cultured cell lines were reverse transcribed using the SuperScript first-strand synthesis system for RT-PCR (Invitrogen).

2.4. Primers

The genes investigated in this study were *N*-acetylglucosamine-6-*O*-sulfotransferase (Gene accession number: AB014679), protein tyrosine phosphatase receptor M (Gene accession number: X58288), G protein-coupled receptor 49 (Gene accession number: AF062006) and KIAA1099 protein (Gene accession number: AB029022). Primers were designed for each gene using the Primer3 tool provided by the JustBio web-based bioinformatics resource (www.justbio.com) and are listed in Table 1. DNA sequence analysis was carried out to confirm the identity of each amplicon.

Table 1
PCR primer nucleotide sequences used for the application of gene sequences

Gene	Forward primer	Reverse primer	Size (bp)	Melting peak (°C)
GN6ST	tccaagcctt-tcgtggtatc	cttttaga-gacggggct-tcc	201	89.4±0.7
PTPRμ	tggacacg-cacaatct-gaat	gagctgcc-cagtctga-tagg	203	86.9±0.7
HG38	tgttgctcttc-accaactgc	ctcaggct-cacca-gatcctc	196	81.4±0.5
KIAA1099	agtc-gaatggc-caactatcg	aacgtcctg-gaa-gaccctct	198	86.8±0.6

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