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ORIGINAL ARTICLE

Detection and analysis of tumour biomarkers to strengthen the diagnosis of acute and chronic leukaemias

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Abstract Molecular markers in leukaemia are essential to diagnose, establish prognosis factors and determine the correct treatment of patients; therefore, it is imperative to include molecular biology studies, so that, combined with cytomorphology and immunophenotyping studies, they constitute the differential diagnosis of these neoplasias. It is extremely important to implement a panel of molecular markers that allows us to detect oncogenes derived from chromosomal translocations, genes derived from epigenetic alterations and drug-resistant genes.

A panel of molecular markers that included 11 genes derived from chromosomal translocations BCR-ABL major and minor breakpoints, E2A-PBX1, MLL-AF4, TEL-AML1, PML-RARα, AML1-ETO was standardised; cancer testis antigens (CTA) derived from NY-ESO1 and MAGE-A3 epigenetic alterations and multi-drug-resistant genes ABCB1 and ABCG2. 30 patients diagnosed with leukaemia from Mexico's General Hospital (Hospital General de Mexico) were included. They suffered from acute lymphoblastic leukaemia (ALL) and acute myeloblastic leukaemia (AML); bone marrow mononuclear cells were used, from which RNA was extracted for the synthesis of cDNA and RT-PCR for each of the markers. In acute lymphoblastic leukaemia (ALL), BCR-ABL biomarkers expressed under 30% (3/10), E2A-PBX1 10% (1/10), ABC-B1 80% (8/10), and ABC-G2 60% (6/10). Patients with acute myeloblastic leukaemia (AML) expressed 30% PML-RARα (3/10),

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40% ABC-B1 (4/10), and 10% ABC-G2 (1/10). Lastly, in patients with chronic myeloid leukaemia (CML), BCR-ABL was over 100% (10/10), ABC-B1 20% (2/10), and ABC-G2 50% (5/10). The presence of transcripts from chimeric genes minor BCR-ABL and E2A-PBX1 in ALL; PML-RARα in AML; and major BCR-ABL in CML, confirms the importance that the panel of molecular markers has in strengthening the diagnosis and prognosis of these conditions.

PALABRAS CLAVE
Leucemia;
Marcadores moleculares;
RT-PCR

Detección y análisis de biomarcadores tumorales como fortalecimiento en el diagnóstico de leucemias agudas y crónicas

Resumen Los marcadores moleculares en leucemias son fundamentales para el diagnóstico, el establecimiento de factores pronósticos y la determinación del tratamiento adecuado para los pacientes; por tanto es obligado incluir los estudios de biología molecular, para que aunado a los de citomorfología e Inmunofenotipo, conformen el diagnóstico diferencial de estas neoplasias. Es de gran importancia implementar un panel de marcadores moleculares que permita detectar oncogenes derivados de translocaciones cromosómicas, genes derivados de cambios epigenéticos y de resistencia genes a drogas.

Se estandarizó un panel de marcadores moleculares que incluyeron 11 genes derivados de translocaciones cromosómicas BCR-ABL rompimiento mayor y menor, E2A-PBX1, MLL-AF4, TEL-AML1, PML-RARα, AML1-ETO; los antígenos testiculares de cáncer (ATC) derivados de cambios epigenéticos NY-ESO1 y MAGE-A3 y genes de resistencia multidrogas ABCB1 y ABCG2. Se incluyeron 30 pacientes con diagnóstico de leucemia del Hospital General de México, de los tipos, leucemia aguda linfoblástica (LAL) y mieloblástica (LAM) así como leucemia mieloide crónica (LMC); se utilizaron células mononucleares de médula ósea, a las cuales se les extrajo RNA para la posterior síntesis de cDNA y RT-PCR para cada uno de los marcadores. En leucemia linfoblástica aguda (LAL) se expresaron los biomarcadores BCR-ABL menor 30% (3/10), E2A-PBX1 10% (1/10), ABC-B1 80% (8/10), y ABC-G2 60% (6/10). Los pacientes con leucemia mieloblástica aguda (LAM) expresaron PML-RARα 30% (3/10), ABC-B1 40% (4/10), y ABC-G2 10% (1/10). Finalmente, en pacientes con leucemia mieloide crónica (LMC) se encontró BCR-ABL mayor 100% (10/10), ABC-B1 20% (2/10), y ABC-G2 50% (5/10). La presencia de los transcritos de los genes quiméricos BCR-ABL menor y E2A-PBX1 en LAL; PML-RARα en LAM; y BCR-ABL mayor en LMC, confirma la importancia del panel de marcadores moleculares en el fortalecimiento del diagnóstico y pronóstico para dichos padecimientos.

Introduction

Over the last few years, there has been major progress in the understanding of the molecular mechanism associated with normal haematopoiesis and with the development of haematological neoplasias.[1,2] The molecular alterations in genes that control cell differentiation programs, such as proto-oncogenes and tumour suppressor genes, derive in the loss of homeostasis regulation in haematopoietic tissue and promote the development of leukaemia.[3] There are currently over 50 different chromosomal alterations associated with leukaemia.[4–6] The most common damage mechanisms are the balanced chromosomal translocations. A number of times, there are genes involved in chromosomal translocations in which the products are transcription factors that control differentiation mechanisms in haematopoietic tissue precursor cells.[5–7] These transcription factors are responsible for the malignant transformation, and they are essential elements in the design of alternative treatment plans.[8,9] Other molecular alterations that take place in the development of leukaemia are those called point mutations, which damage the mechanisms that regulate cell proliferation, apoptosis and differentiation in haematopoietic precursors.[10,11] Lastly, there are additional alterations that do not affect the information held in the DNA nucleotide sequence, which are associated with epigenetic events; that is to say, they lead to DNA hypermethylation or to abnormal histone acetylation, affecting the transcriptional availability of proto-oncogenes and suppressor genes.[12,13] Mexico does not have a tumour marker panel that could be used to perform the diagnosis, prognosis and follow-up for leukaemia, both acute and chronic, by means of molecular methods such as the RT-PCR. Our laboratory analysed the expression of 11 tumour markers in mononuclear cells from 30 patients with leukaemia. The genes under analysis are involved in the proliferation, differentiation, epigenetic