

OCT embedded sections of pathological specimens as a source of high quality RNA for reverse transcriptase/polymerase chain reaction

J A E Irving, G Cain, A Parr, M Howard, B Angus, A R Cattan

Abstract

OCT embedded cryostat sections of stored pathological specimens of non-Hodgkin's lymphoma were used to provide RNA. After reverse transcription to produce cDNA, the polymerase chain reaction was performed with primers for standard and variant forms of the CD44 molecule. Using Southern transfer and hybridisation with a probe specific for exon 4 of the CD44 gene, both standard and variant forms were visualised by autoradiography. This method was shown to be applicable to other gene products by using primers specific for the *abl* and *bcr* genes. This technique permits retrospective analysis of RNA from small amounts of stored pathological samples.

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Keywords: RNA, RT-PCR, cryostat sections, CD44.

CD44 is a ubiquitous cell surface glycoprotein that serves as an adhesion molecule in cell-substrate and cell-cell interactions, including lymphocyte homing, haematopoiesis, cell migration, and metastasis.^{1,2} The molecule exists as a number of isoforms, generated by alternative splicing of 10 variant exons.³ The function and regulation of CD44v molecules is poorly understood but it is known that there is differential expression of variant exons by various epithelia.⁴ The CD44 molecule expressing variant 6 (exon 11) seems to relate to tumour progression, particularly the metastatic potential of some cancers.⁵ In retrospective analysis of breast cancer CD44v6 expression was correlated with poor overall survival.⁶

Here, we report the use of cryostat sections as an alternative source of high quality RNA for visualising CD44 and its variants in non-Hodgkin's lymphoma by reverse transcriptase/polymerase chain reaction (RT-PCR).

Methods

ISOLATION OF RNA

Five pooled cryostat sections (20 µm) were stored frozen at -20°C in a GITC (guanidine isothiocyanate) solution. After thawing, samples were homogenised using a battery operated disposable mortar and pestle (Anachem, Luton, UK). The method of Chirgwin *et al*⁷ was used to isolate RNA after caesium chloride ultracentrifugation, phenol/chloroform ex-

traction and ethanol/salt precipitation. Denaturing formaldehyde gel electrophoresis was used to assess the quality of the RNA.

cDNA PREPARATION AND POLYMERASE CHAIN REACTION

The method of Cross *et al*⁸ was used to reverse transcribe about 5 µg RNA, using random hexamers and M-MLV (Life Technologies, Paisley, Scotland). PCR primers and reaction conditions were as described by Matsumura and Tarin⁵ for CD44 and by Cross *et al*⁹ for *bcr* and *abl*. PCR products were analysed by gel electrophoresis using 3% Nusieve agarose (FMC Bioproducts, Rockland, Maine, USA) and photographed after staining with ethidium bromide.

BLOTTING AND PROBING

After Southern blotting onto Genescreen (NEN, Boston, Massachusetts, USA), filters were hybridised with end-labelled probes as described by Matsumura and Tarin⁵ and developed as autoradiographs using standard techniques.

Results and Discussion

Denaturing gel electrophoresis of purified RNA showed distinctive 28S and 18S RNA which can be routinely extracted from frozen sections of lymph nodes, currently dating back three years (figure, panel A). Triplicate 20 µm sections were used for RNA extraction initially; however, we were unable to obtain sufficient RNA from many samples. As a consequence, we now routinely pool five sections per sample which has permitted successful RT-PCR analysis of CD44 expression and subsequent analysis by Southern blotting of CD44 variants in 28 of 30 samples (figure, panels B and C). We have been able to show that RNA extracted by this method is suitable for the analysis of other gene products—for example, *bcr* and the house-keeping gene, *abl* (figure, panel B).

This technique permits retrospective analysis of small amounts of stored pathological samples. Results may then be correlated with clinical outcome. This may be particularly important for CD44 expression as there is an ever increasing list of malignancies in which CD44v expression, particularly variant 6, is a marker of metastasis and thus prognosis. Although immunocytochemical techniques with specific

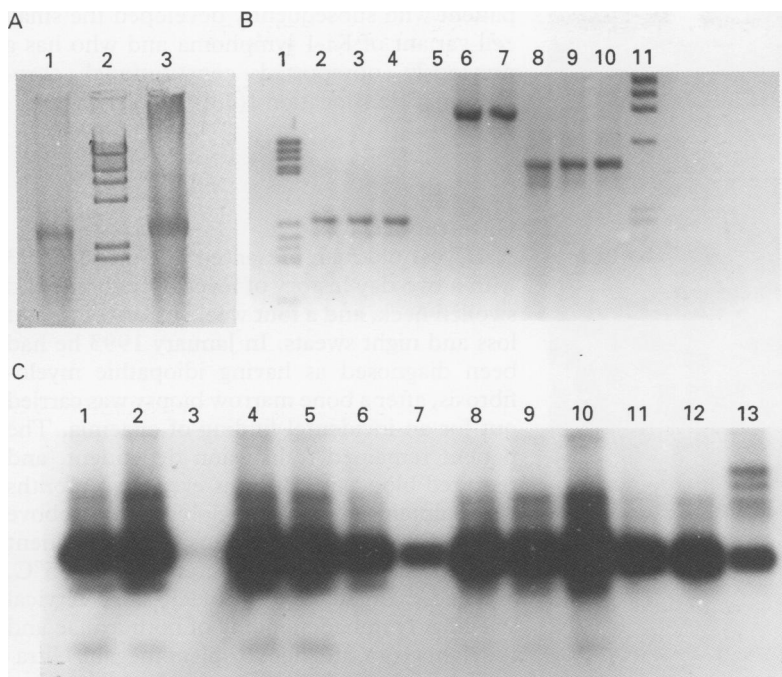
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(A) Formaldehyde agarose electrophoretic gel of RNA. Lanes 1 and 3, 3 μ g RNA extracted from OCT embedded sections of samples from two patients with non-Hodgkin's lymphoma; lane 2, λ HindIII markers. (B) Agarose electrophoretic gel of PCR products. RNA was extracted from three different samples from patients with non-Hodgkin's lymphoma as outlined in the methods section and reverse transcribed into cDNA. An aliquot of each was used as a substrate for PCR using three different primer sets. Lanes 2-4, abl; lanes 5-7, bcr; lanes 8-10, CD44; lanes 1 and 11, pBR322 AluI and ϕ X174 HaeIII, respectively, as base pair markers. (C) Analysis of CD44 isoforms in non-Hodgkin's lymphoma samples after PCR and hybridisation with a radiolabelled oligoprobe to a non-variant part of the molecule (exon 4). Note: lane 3 in panel A, lanes 3, 6 and 9 in panel B and lane 9 in panel C represent RNA, PCR products and blot of the same frozen non-Hodgkin's lymphoma sample, respectively.

antibodies can detect variant expression in cryostat sections, the PCR method may detect

small numbers of CD44v positive cells, not apparent by antibody staining because of a lack of sensitivity or masking of the antigenic sites.¹⁰ Combining the method described here with immunohistochemistry on sequential sections may permit a more detailed analysis of cell phenotype. In addition, PCR analysis followed by Southern blotting gives additional information on the fingerprint pattern of variant expression, which may have biological implications.

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Small cell variant of Ki-1 lymphoma associated with myelofibrosis and a novel constitutional chromosomal translocation t(3;4)(q13;q12)

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Abstract

An unusual case of small cell variant of Ki-1 non-Hodgkin's lymphoma diagnosed one year after an original diagnosis of idiopathic myelofibrosis is reported. On the second occasion, the patient presented with fever, lymphadenopathy and hepatosplenomegaly. A lymph node biopsy specimen confirmed a diagnosis of small cell variant of Ki-1 lymphoma. A repeat bone marrow biopsy specimen showed myelofibrosis with no evidence of lymphomatous infiltration, but cytogenetic studies on blood, bone marrow and skin

fibroblasts revealed a novel chromosomal translocation t(3,4)(q13;q12).

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Keywords: Ki-1 lymphoma, myelofibrosis.

Small cell Ki-1 lymphoma is a variant of Ki-1 anaplastic large cell lymphoma (ALCL), which has been described recently.¹ The specific chromosomal translocation t(2;5)(p23;q35) has been a consistent finding in association with both variants of Ki-1 lymphoma.¹⁻³ Lymphoma is a recognised, albeit uncommon, cause of myelofibrosis. However, idiopathic myelo-