Original articles

Activation of Lyt-2 associated with distant upstream insertion of an SL3-3 provirus

Donald S. Anson, Kristie Clarkin, and Robert Hyman

Department of Cancer Biology, The Salk Institute, P. O. Box 85800, San Diego, CA 92186-5800, USA

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Abstract. Two Lyt-2 + mutants of the T-cell lymphoma SL12.4.10 were selected by fluorescence activated cell sorting. Both mutants expressed Lyt-2 (CD8 α-chain) but not Lyt-3 (CD8 β-chain). Derivatives of one Lyt-2 + mutant that expressed Lyt-3 could be isolated by sorting for Lyt-3 + cells. Southern blotting analysis indicated that both mutants had structural rearrangements within or immediately 3' of the Lyt-3 gene, accompanied by demethylation of at least one Hpa II site within the Lyt-2 gene. Gene cloning analysis of one mutant demonstrated that the structural rearrangement was due to insertion of an SL3-3 provirus 35 kb 5' to the Lyt-2 gene. It is likely that Lyt-2 gene activation is a direct or indirect consequence of proviral insertion at this site.

Introduction

The activation of cellular genes by the integration of a provirus adjacent to the gene in question is now well documented (Varmus 1984; Nusse 1986; Keshet et al. 1991). The integration event can give rise to transcription of the cellular gene either by the provirus acting as a promoter element or as a transcriptional enhancer for the cellular gene's own promoter. In the latter case, the provirus may be found either upstream or downstream of the transcribed sequence. In most cases, the provirus is found within a few kilobases (kb) of the promoter. In some instances, however, the site of proviral integration may be 17–270 kb from the activated cellular gene (Corcoran et al. 1984; Peters et al. 1989; Lazo et al. 1990). This is of interest with respect to the normal control of transcription via enhancer elements. Generally, enhancers act in concert with the closest promoter, although the enhancer/promoter orientation and distance are variable (Serfling 1985; Ptashne 1988). However, the activation of a particular promoter by an enhancer may depend on additional specific regulatory elements (Choi and Engel 1988).

Endogenous proviruses are dispersed throughout the mouse genome (Frankel et al. 1989). Some are integrated near genes coding for cell surface molecules (Meruelo et al. 1983; Wejman et al. 1984; Frankel et al. 1989). The significance of proviral integration for expression of these genes in vivo is uncertain. Examples of the alteration of gene expression caused by retroviral integration in vivo are known (Stoye et al. 1988; Keshet et al. 1991). Spontaneous or induced T-cell lymphomas show complex patterns of proviral integrations (Canaani and Aaronson 1979; Quint et al. 1981; van der Putten et al. 1981; Herr and Gilbert 1983; Mucenski et al. 1988; Ihle et al. 1989). It is thought that the activation or repression of cellular genes may be one consequence of these proviral integrations (Cooper and Lane 1984; Nusse 1986; Bear et al. 1989; van Lohuizen et al. 1989). Here, we report the isolation of somatic cell mutants of a mouse T-cell lymphoma that have activated expression of the gene coding for the cell surface molecule Lyt-2 (CD8 α-chain). We present evidence that the activation event is associated with a proviral insertion 35 kb 5' to the Lyt-2 gene and with the demethylation of one or more sites within the Lyt-2 gene.

Materials and methods

Cell lines. SL12.4.10 is an Lyt-2 + clone of the tissue culture adapted AKR/J lymphoma SL12.4 which was obtained from a spontaneous tumor of an AKR/J mouse (MacLeod et al. 1984). Lyt-2 + mutants of SL12.4.10 were obtained by fluorescence activated cell sorting (FACS) for Lyt-2 + cells (Hyman et al. 1982). Mutant 1 was isolated after mutagenesis with ethylmethane sulfonate. Cells (5 x 10⁶) were treated with 0.7 mg/ml ethylmethanesulfonate for 18 h, grown for 7 days and then sorted for the most fluorescent 0.3% of cells. These cells were grown and the sorting procedure repeated. A detectable Lyt-2 + population was seen when the cells were sorted a third time. An Lyt-2 + clone was isolated by limit dilution cloning. Mutant 2 was isolated by an
on a different chromosome than the gene coding for Lyt-2, the relative intensities of the Thy-1 bands should serve as a measure of the amount of DNA loaded/lane and as a control for relative differences between lanes in the amount of DNA transferred. Values are expressed relative to the parental cell line as 1.0.

Results

Isolation of Lyt-2+ mutants. The SL12.4.10 parental cell line expresses neither Lyt-2 (CD8 α chain) nor Lyt-3 (CD8 β chain) on its cell surface (Fig. 1) and accumulates neither Lyt-2 nor Lyt-3 mRNA’s (Fig. 2). Two Lyt-2+ mutants were isolated by FACS as described in Materials and methods. Mutant 1 was isolated after treatment of SL12.4.10 with ethylmethane-sulfonate, while mutant 2 was isolated from an independent selection of SL12.4.10 not subjected to mutagenesis.

By flow cytometry, both mutants expressed Lyt-2 antigen on their cell surface at 30–100× background levels (Fig. 1). Lyt-3 expression on the cell surface of the mutants ranged from undetectable to 2× background (Fig. 1). The level of accumulation of Lyt-2 and Lyt-3 mRNA as determined by northern blotting reflected the level of cell surface expression (Fig. 2). Nuclear run-on transcription analysis confirmed that the level of mRNA as determined by northern blotting reflected gene transcription (data not shown). Analysis of the DNA content/cell of the parental and mutant cell lines (Crisman and Steinkamp 1982) indicated that the parental cell line and mutant 1 were pseudodiploid, while mutant 2 was pseudotetraploid.

The mutations defined by these two cell lines might act in trans position or in cis position to the Lyt-2 gene. Somatic cell hybrids between mutant 1 (Lyt 2.1+) and the BALB/c (Lyt 2.2-) T-cell lymphoma SL12.4.10 were examined for expression of antigens coded for by the respective Lyt-2 alleles (Table 1). All hybrids maintained expression of the Lyt 2a allele characteristic of mutant 1 (although at a somewhat reduced level) and no hybrid showed expression of the Lyt 2b allele. No restriction fragment length polymorphism (RFLP) that differentiates between Lyt-2 alleles is known (Liaw et al. 1986; Youn et al. 1988) and we did not observe any structural polymorphisms for Lyt-2 between AKR/J and BALB/c mice when a number of restriction endonucleases were surveyed. We were able to demonstrate that all hybrids retain the closely linked Lyt-3 gene of each parent (Table 1). Thus, it is very unlikely that the failure of these hybrids to express Lyt 2.2 reflects selective loss of the Lyt 2b gene. We conclude, therefore, that the mutation defined by mutant 1 acts in cis position to activate Lyt-2 expression.

Gene structure of mutant cell lines. The gene structure of the Lyt-2 and Lyt-3 genes in the parental and mutant