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Viral Transduction of Host Genes in Naturally Occurring Feline T-Cell Leukaemias: Transduction of *myc* and a T-Cell Antigen Receptor β-Chain Gene

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A. Introduction

Feline leukaemia virus has been a particularly useful tool in cancer research since many of the naturally occurring tumours associated with this virus group have yielded recombinant retroviruses containing host-derived oncogenic information. The prevalence of transduction as an oncogenic mechanism was seen first in multicentric fibrosarcoma, a relatively rare tumour in FeLV-infected cats (Hardy et al. 1982; Besmer 1984). In a significant percentage of cases of this disease, oncogene-containing feline sarcoma viruses have been identified. More recently, we and others have found that in the more common FeLV-associated neoplasm, thymic lymphosarcoma, viral capture of the c-*myc* gene can occur (Neil et al. 1984; Levy et al. 1984; Mullins et al. 1984). Since the oncogenes carried by feline sarcoma viruses do not include *myc*, these reports provided the first evidence that *myc* may be a target for oncogenic activation by FeLV.

Further study of feline tumours revealed that c-*myc* could be affected either by viral transduction or by proviral insertion into the cellular gene locus (Neil et al. 1984, 1987; Forrest et al. 1987) although the majority of field-case tumours showed neither of these features. To gain more information on the cellular origin of the feline lymphoid tumours and to search for some distinguishing feature of those with activated *myc* genes, we undertook an analysis of the state of rearrangement and expression of the genes encoding α and β chains of the T-cell antigen receptor. In the course of this analysis we discovered a novel FeLV provirus in which a full-length β-chain gene had been incorporated into the viral genome (Fulton et al. 1987). The present report describes this novel provirus and considers its possible significance in leukaemogenesis.

B. T-Cell Antigen Receptor Gene Rearrangements and Expression in Feline Leukaemias

Analysis of feline T-cell receptor genes was performed with cDNA probes derived from the human α (pJα6) and β (pB400) genes (Collins et al. 1985 b); these were kindly provided by Michael Owen (ICRF Tumour Immunology Labs, London). We found these probes to be strongly cross-reactive with the feline genes and their transcripts, although hybridisation was stronger with the β-chain than with the α-chain probe. For this reason, only the β-chain probe has been used to assess gene rearrangement.

The overall gene arrangement and transcript sizes appear similar for feline and human genes. Thus, as in the human and the murine Cβ loci (Gascoigne et al. 1984; Malissen et al. 1984; Sims et al. 1984) the feline Cβ coding sequence appears to be tandemly duplicated in germ-line DNA. Also, some of the feline tumours examined displayed rearrangement of both Cβ alleles with two distinct transcript sizes of 1.2 and 1.4 kb, which by analogy with human genes (Collins et al.

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Table 1. Rearrangements of T-cell antigen receptor β-chain gene in feline leukaemias

1. Thymic and multicentric lymphosarcomas			
(a) *myc* gene involved		(b) No detected *myc* rearrangement	
FeLV v-*myc*	T3 +[a]	FeLV+ve	84904 −[c]
	F422 +[a]		89407 +
	T17 +[b]		86800 +
	84793 +		T14 +
	T11 +		Q109 −
c-*myc*	T8 +	FeLV-ve	86503 −
Rearranged	T7 +		89960 +
	T5 +		T16 −
			T20 −
2. Other tumours			
Spleen lymphosarcoma (FeLV-ve)			T9 +[d]
Alimentary		FeLV-ve	87416 −
Lymphosarcomas[e]		FeLV+ve	87655 −
			75800 −
			83029 −

Rearrangements of β-chain genes of T-cell antigen receptor were assessed by Southern blot hybridisation analysis. The Cβ probe hybridises to a 18 kb *Eco*RI fragment in germ-line DNA of most cats. Where possible, digests of DNA from tumour and uninvolved tissues (usually kidney) were run side by side. In some cases both germ-line bands were rearranged. In most cases the pattern of rearrangement was consistent with a monoclonal tumour outgrowth. However, tumour 86416 showed evidence of a bi-clonal nature. For all cases examined so far Northern blot analyses show that both α- and β-chain transcripts are expressed in cases with rearranged β-chain genes.
[a] All tumours induced experimentally by inoculation of GT3 and F422 viruses were also positive for gene rearrangement.
[b] Amplified due to FeLV transduction.
[c] *pim*-1 gene rearranged.
[d] c-*myc* amplified (Neil et al. 1984).
[e] Assumed to be of B-cell origin.

1985a) correspond to abortive (D-J-C) and successful (V-D-J-C) joining events, respectively. The results of a survey of β-chain rearrangement are given in Table 1. The conclusions both from these data and from those of Northern blot analyses for the expression of α- and β-chain transcripts are that feline thymic tumours are heterogeneous with respect to maturity as assessed by T-cell receptor gene rearrangement and expression. However, the tumours involving c-*myc* activation, either by transduction or by proviral insertion, represent a homogeneous subset with mature characteristics (expressing both α- and β-chain transcripts).

C. A FeLV Provirus Containing a β-Chain T-cell Antigen Receptor Gene

Tumour T17 showed an anomalous pattern of β-chain mRNA both in size (>6 kb) and in abundance. Furthermore, DNA blots showed gross amplification of sequences hybridising to the human Cβ probe. Further Southern blot hybridisation analysis of tumour T17 showed that the amplified sequences could be resolved into a single, intensely hybridising fragment if digestion was performed with any of the enzymes which characterise the FeLV LTR (*Kpn*I, *Sma*I, *Pst*I and *Hin*cII) (Fig. 1). These data provide indirect but persuasive evidence that the amplification of β-chain sequences in tumour T17 was due to their presence *within* multiple FeLV proviruses.

Cloning was undertaken to isolate the novel proviral structures from tumour T17. From a library of size selected (15–23 kb), *Eco*RI-digested tumour DNA in lambda EMBL 4 we selected recombinants with various probes, including the human Cβ cDNA clone, FeLV v-*myc*, FeLV *env* and FeLV LTR. The clones we have isolated correspond to the proviruses containing Cβ sequences, proviruses containing v-*myc*, FeLV helper-type proviruses and the normal cellular loci of c-*myc* and Cβ.

Our initial efforts have focussed upon characterising the FeLV proviruses containing the Cβ-hybridising sequences. A 1.9-kb fragment containing the entire hybridising sequence was sequenced and found to contain a 1.2-kb host-derived sequence insert including the intact coding sequence of a β-chain T-cell receptor gene (Fulton et al. 1987; see Fig. 1). The β-chain gene appears to have undergone productive rearrangement since sequences clearly identifiable as those of Vβ, Dβ, Jβ and Cβ origin are seen. Intron sequences are missing, however, as might be expected if the sequence has been transmitted as part of a retroviral replication

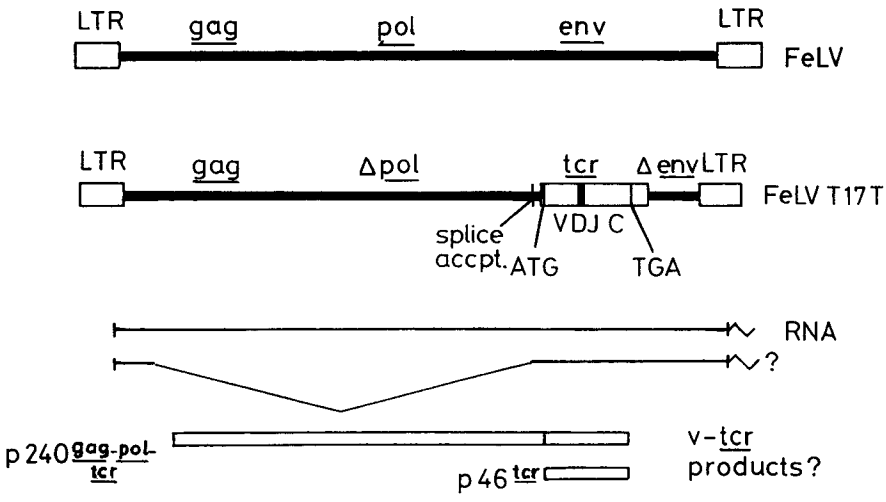

Fig. 1. Structure of the FeLV provirus (FeLV T17T) containing a T cell antigen receptor β-chain gene. Within the T17T provirus, the v-*tcr* gene replaces the 3′ end of *pol* and much of the *env* gene. The host-derived insert contains the complete coding sequence for a β-chain gene product including N-terminal signal peptide and variable, diversity, joining and constant region-derived sequences. The 3′ end of the insert appears to be coincident with the β-chain gene AATAAA sequence at the end of the 3′ untranslated region. As shown underneath, two modes of expression appear to be possible for the as yet uncharacterised v-*tcr* product. From genomic RNA read-through from *gag* and *pol* into *tcr* appears possible since the reading-frames are coincident. Alternatively, the splice acceptor site usually employed to generate *env* mRNA may serve to produce an RNA encoding a full-length β-chain product which is not fused to any viral protein

unit. The host-derived sequence replaces the 3′ end of *pol* and much of the FeLV *env* gene. The recombination junction is 7 base pairs upstream of the ATG, marking the beginning of the β-chain open reading frame which begins with the characteristic signal peptide for membrane insertion. The 3′ end of the host insert appears to be coincident with the polyadenylation signal (AATAAA). In this context the host-derived sequence (which we have designated v-*tcr*) could be expressed as a protein in two different ways. Firstly, since the *pol* reading frame is coincident with that of v-*tcr*, the gene may be expressed as a large fusion protein including *gag*, *pol*, and *tcr* sequences. Alternatively, the proximity of the splice acceptor site for the *env* mRNA means that a spliced subgenomic RNA could direct the synthesis of a v-*tcr* product which is neither truncated nor fused to viral sequences (Fig. 1). We are at present investigating these possibilities.

D. The *myc*-Containing Provirus in Tumour T17

Rather less information is available at present regarding the *myc*-containing provirus from tumour T17. Although EMBL 4 phage clones were readily obtained, full-length sub-clones have proved impossible to obtain thus far. Possible "poison" sequences have not yet been located in the provirus or its flanking sequences, but cloning in segments into plasmid vectors has allowed us to isolate possibly all the proviral structure. Since T17 represents the first recorded example of a "double transduction" event in which both host genes are present on separate proviruses, we wish to discover whether these recombinants arose independently or whether one recombinant provirus may have arisen from the other. Initial mapping suggests that the *myc* gene replaces *env*, as in the v-*tcr*-containing virus, however we do not yet know the precise 5′ and 3′ junctions.

E. Discussion

Although FeLV *myc* recombinant viruses appear to be potent initiators of tumour development, several features led us to consider that the FeLV v-*myc* genes may be insufficient for full neoplastic development. The first factor we considered is the latent

period for FeLV *myc* virus-induced tumour development, which is shorter than that for helper FeLV but longer than that for many other v-*onc*-containing retroviruses. Also, analysis of integrated proviruses by Southern blot hybridisation with FeLV or v-*myc* probes indicated that even the short latency FeLV *myc* tumours represent monoclonal or oligoclonal outgrowths of virus-infected cells. Furthermore, these tumour cells could be established readily in culture in the absence of exogenous sources of interleukin 2 (IL-2), although such transformed cell lines could not be obtained in vitro even after a series of attempts to infect isolated T cells or bone marrow cell cultures (Onions et al. 1987).

While these phenomena may also be explained in other ways, we consider that the sum of the evidence points to secondary oncogenic events in vivo which we cannot so far reproduce in vitro. The finding here of a novel provirus containing a T-cell antigen receptor gene in the same tumour as a v-*myc* gene suggests a possible secondary oncogenic factor for this one case. In the majority of cases which do not show such proviruses we must seek other explanations. However, the observation that all of the tumours involving direct *myc* activation are of mature T-cell phenotype may provide a useful clue.

The oncogenic properties of v-*tcr* have not yet been tested by in vivo experiments. The primary tumour from T17 is no longer available and was in any case a very poor virus producer. We have therefore had to resort to transfection experiments to reconstruct virus complexes for inoculation into cats; these experiments are in progress. Predictably perhaps, initial experiments have shown no transforming potential of v-*tcr* for fibroblastic cells. Transfections into mature human T cells have been undertaken to discover whether the v-*tcr* gene product(s) can interact with human α chain and other T-cell receptor components and lead to membrane transport of the complex. At the same time, we will monitor any disturbance in growth or responsiveness to external stimuli (e.g. lectins, phorbol esters) which may give clues to the mode of action of v-*tcr*.

Our initial hypothesis was that v-*tcr* might cause constitutive activation of the antigen receptor in the absence of external antigen (Fulton et al. 1987). The rationale for this model was that the transmembrane region of v-*tcr* has a nonconservative change (met → lys) relative to the human and mouse Cβ sequences. Thus, in a manner akin to the proposed mechanism of activation of the *neu* gene (Bargmann et al. 1986), altered conformation might mimic the presence of extracellular ligand. This model now appears less likely for v-*tcr* in view of our finding that the cellular Cβ locus cloned from tumour T17 has the same transmembrane sequence as the viral gene (J.N. and R.F., unpublished results). The difference which we recorded (Fulton et al. 1987) may therefore be a species-specific change.

These results leave both the oncogenic significance and the possible modes of action of v-*tcr* as open questions. We will have to await the outcome of in vivo experiments for the answer to the first question. We may then have to address the possibility that the immunological specificity carried by v-*tcr* is the key to its oncogenic function. Recognition of a host or viral antigen seems possible, although the associated α chain might then be expected to play a contributory role. If self-reactivity is involved, we may speculate further that the v-*tcr* specificity would normally have been suppressed during thymic education of lymphocytes where specificity for host-MHC plus foreign antigen is learned (Bevan 1981). This self-reactivity may have been augmented in T17 by the retroviral capture of the important part of the immune effector involved in recognition. We might also propose that the role of v-*myc* in this case is to rescue (immortalise) a cell clone with autologous reactivity and "self-driven" proliferative capacity. Whatever the explanation, it is our hope that v-*tcr* may contribute in a wider sense to our understanding of normal and neoplastic T-cell growth.

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