C-Myc Activation in Spontaneous Rat Immunocytomas Containing a 6;7 Chromosomal Translocation

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The Lou/Wsl rat immunocytoma (RIC) is a plasma cell tumor (Bazin et al, 1972; for review, see Bazin, 1986), and histologically resembles the mouse plasmacytoma. Tumors arise spontaneously in the ileocecal lymph nodes in approximately 28% of rats of the Lou/Wsl strain, and are detectable at about one year of age. The tumors kill the animals approximately one month after becoming palpable. Although the most frequently secreted myeloma protein is IgE, all isotypes and Bence-Jones proteins are secreted. In addition to the genetic predisposition of the Lou/Wsl strain, both parasites and sex have been postulated as factors in immunocytoma development (Bazin, 1985).

Similar to the non-random chromosomal translocations identified in Burkitt's lymphoma (BL) and mouse plasmacytoma (MPC) (reviewed in Klein and Klein, 1985), it has been shown that the RIC contains a consistent chromosomal translocation between chromosomes 6 and 7 (Wiener et al, 1982). The 6;7 translocation has been identified in all 15 RICs examined cytogenetically (Table 1) (Wiener et al, 1982; Szeles and Wiener, unpublished results). In their paper describing the cytogenetics of the RIC, Wiener et al (1982) noted that the RIC translocation resembles the MPC, and hypothesized that the translocation in the RIC would involve the same loci as in the 12;15 MPC translocation.

By utilizing rat/mouse somatic cell hybrids (Szpirer et al, 1984), we have shown that the same genes involved in the 12;15 MPC translocation, c-myc and IgH, are located on the chromosomes involved in the RIC translocation (Table 2). The c-myc gene has been localized to chromosome 7 (Sumegi et al, 1983) and the immunoglobulin heavy chain cluster (IgH) to chromosome 6 (Pear et al, 1986). Cytogenetic studies of 15 RICs have not identified any tumors which display a translocation between the chromosomes containing the c-myc (7) and Ig kappa locus (4) (Szeles and Wiener, personal communication). We are in the process of localizing the rat Ig lambda locus.

Cleavage of rat genomic DNA with EcoR1 generates a 17 kb c-myc-specific fragment which contains all 3 exons and 5' and 3' flanking sequences. Hybridization of the human c-myc 3rd exon probe, pMC41-3RC (Dalla-Favera et al, 1982) to EcoR1 digested DNA from 15 RICs showed that the c-myc-carrying fragment is

Table 1: Summary of Lou/Wsl rat immunocytomas

TUMOR	TRANSLOC TYPE	COPIES t6;7	IG SECR	SIZE OF ECORI REARR C-MYC	IG COMIG
IR9	t(6;7)	1	BJ	None	*
IR27	t(6;7)	1	gamma1/K	>23	*
IR33	t(6;7)	1	gamma2a/K	>23	*
IR49	t(6;7)	1	BJ-K	>23	*
IR50	ND	ND	epsilon/K	>23	*
IR72	t(6;7)	1	epsilon/K	>23	*
IR74	t(6;7)	1	none	>23	*
IR75	t(6;7)	1	epsilon/K	12	S _u
IR88	t(6;7)	1	epsilon/K	>23	*
IR89	t(6;7)	1	epsilon	>23	ND
IR209	t(6;7)	1	gamma2a/K	12	*
IR222	t(6;7)	1	epsilon	18	*
IR223	t(6;7)	2	BJ	20	epsilon
IR241	ND	ND	gamma1/K	>23	*
IR304	ND	ND	gamma2a/K	None	*

Notes: ND-Not Determined; *-No Co-migration detected; TRANSLOC-Translocation; Ig SECR- Secreted Immunoglobulin; K-Kappa; BJ-Bence-Jones; Ig COMIG-Immunoglobulin Co-migration

rearranged in 13 of these tumors (Table 1 and Figure 1). As shown in Table 1, the rearranged c-myc-containing EcoRI fragment is greater than 23 kb in the majority of these tumors. In two tumors, IR75 and IR209, the rearranged c-myc carrying EcoRI fragment is approximately 12 kb. The rearranged c-myc-carrying EcoRI fragments from both of these tumors have been cloned into EMBL4.

Analysis of the c-myc breakpoints in the IR75 and IR209 has shown that the breakpoints occur upstreams of the proximal c-myc promoter, approximately 800 bps in the IR75 and 600 bps in the IR209. This is upstreams of the most frequent c-myc breakpoints in both MPC and BL (reviewed in Cory, 1986). Sequence analysis has demonstrated that the IR75 has undergone multiple rearrangements which juxtapose part of the switch mu region with the c-myc gene in opposite transcriptional orientations. Upstreams of the juxtaposed switch mu sequences are sequences derived from upstreams of the switch gamma1 region. Thus, like both BL and MPC, the translocation in the IR75 has juxtaposed c-myc and an Ig loci. Since the chromosome 6 breakpoint is located at 6q3.2, this is most likely the location of the rat IgH locus. Likewise, the c-myc gene is located at 7q3.

Table 2: Chromosomal Location of C-Myc, IgH, and Ig Kappa Loci

LOCUS	RAT CHROMOSOME
C-MYC	7 (Sumegi et al, 1983)
IgH	6 (Pear et al, 1986)
IgKappa	4 (Perlmann et al, 1985)

We have utilized genomic hybridization to determine the c-myc breakpoint in 13 other RICS (Figure 2). Two RICS, IR9 and IR304, do not show rearrangement with EcoR1. Further chromosome walking has demonstrated that these two tumors are not rearranged within at least 23 kb of the proximal c-myc promoter. As shown in Figure 2, the c-myc-associated breakpoints are clustered within a 900 bp BamH1 fragment which contains the proximal c-myc promoter and approximately 700 bps of 5' flanking sequence. This is the same region in which viral insertion occurs in murine T-cell lymphomas (Corcoran et al, 1984; Li et al, 1984; Selten et al, 1984) and in Moloney virus-induced rat thymomas (Steffen, 1984). The breakpoint in IR49 occurs in the intron between exons 1 and 2; similar to the most common breakpoint in Balb/c MPCs. The breakpoints in IR74 and IR241 occur at least 700 bps upstreams of the proximal promoter. As in BL and MPC, no breakpoints have been identified which interrupt the c-myc coding exons.

Co-migration experiments between the rearranged c-myc-carrying fragment and Ig switch and constant region probes have shown that the translocation in the IR223 has juxtaposed the c-epsilon locus with the c-myc gene. Surprisingly, none of the other 13 analyzed tumors has shown co-migration between the rearranged c-myc and Ig switch and constant region probes. As with the IR75, however, it is possible that rearrangement takes place within other regions of the IgH cluster. Analysis of other cloned RICS should elucidate which Ig regions are involved in the translocation event.

Figure 1: C-myc rearrangement in the RIC. DNA was digested with EcoRI and transferred to nitrocellulose. The filter was hybridized to the 32 P-labeled human c-myc 3rd exon probe, pMC41-3RC (Dalla-Favera et al, 1982). High stringency washings were done in 0,2 X SSC, 0,2% SDS for 1 hr at 65 degrees.

In summary, our studies of the RIC show that in 3 different tumors (BL, MPC, RIC) in 3 different species (human, mouse, rat), nearly identical genetic loci (c-myc, Ig) are juxtaposed via chromosomal translocation. These 3 tumors represent at least two different stages of B cell maturation, and the natural histories and modes of induction of the tumors are very different. The finding that c-myc/Ig juxtaposition occurs in these three tumors suggests that this configuration plays a central role in the genesis of these B cell tumors.

Figure 2: Identification of translocation breakpoints within the 17 kb EcoRI c-myc-carrying fragment in 15 RICs

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