c-myc gene rearrangements involving gamma immunoglobulin heavy chain gene switch regions in murine plasmacytomas

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ABSTRACT

In murine plasmacytomas, the c-myc gene has frequently been found to undergo rearrangement by virtue of a T(12;15) chromosome translocation. The immunoglobulin heavy chain gene switch region (S_{α}) constitutes the target for most of these recombinations particularly in IgA producing plasmacytomas. We sought to identify non- S_{α} c-myc target sites in several IgG producing tumors. The c-myc target in MPC-11 (a BALB/c IgG_{2b} producing plasmacytoma) has been cloned, localized to the Igh-C locus and identified as the γ 2a heavy chain gene switch region ($S_{\gamma 2a}$). Furthermore, by Southern blot hybridization, we have determined that the $S_{\gamma 2b}$ region is the c-myc target in two NZB IgG_{2b} producing plasmacytomas. The potential relation between Ig class expressed and c-myc translocation target is discussed.

INTRODUCTION

We and several other groups of investigators have found a new class of aberrantly rearranged alpha immunoglobulin heavy chain genes in murine plasmacytomas (1-4). A segment of DNA of unknown origin, referred to as NIARD (Non-Immunoglobulin-Associated Rearranging DNA) was found adjacent (5') to the alpha switch region (S_{α}) (2). It was shown that NIARD resides on chromosome 15 in the mouse germline (5,6) while immunoglobulin heavy chain genes are known to reside on chromosome 12. Interestingly, cytogenetic data had previously demonstrated the existence of a T(12;15) chromosome translocation in murine plasmacytomas (7-9). However, it remains to be proved that the cytogenetically observed translocation is the same as the one involving NIARD.

More recently, we and others have found that the mouse c-myc gene lies within the segment of DNA previously termed NIARD (10-15). DNA sequencing, Northern and Southern blotting studies have conclusively shown that the mouse c-myc gene contains at least three exons and that the translocation breakpoint occurred either within the first exon or intron, depending on the specific plasmacytoma (16). The existence of truncated c-myc RNAs in plasmacytomas with NIARD rearrangements (10,13-15) was consistent with these conclusions. In

addition, it was found that the level of myc transcripts was increased (5-20X) in most plasmacytomas (regardless of whether a myc rearrangement had occurred) compared to normal spleen cells (13,14). In addition, the unrearranged myc allele in plasmacytomas has been found to be transcriptionally silent or expressed at a lower level than the translocated allele (15,16).

The experiments presented here were designed to identify NIARD (i.e. c-myc) targets independent of S_{α} . Knowledge of the nature of these c-myc acceptor sites may help in understanding the mechanism by which these translocations occur. The c-myc target in MPC-11, a BALB/c γ 2b producing plasmacytoma, has been cloned and found to be the $S_{\gamma 2a}$ region. Furthermore, Southern blotting experiments indicate that two γ 2b producing NZB plasmacytomas contain an $S_{\gamma 2b}$ associated c-myc rearrangement.

MATERIALS AND METHODS

A 16.5 kb EcoRI fragment containing the rearranged myc sequence in MPC-11 cells was purified by agarose gel electrophoresis and cloned in Charon 4A phage (17). Phage plaques were screened (18) with a plasmid containing myc exon and intron sequences p α 25BH3.4, which has been described previously (2).

Genomic Southern blots were performed essentially as described (19-21).

Figure 1

Restriction endonuclease maps of the normal BALB/c c-myc gene and the rearranged c-myc genes of J558 (16) and MPC-11 tumor cells. Myc rearrangement sites are indicated by arrows. The 5' most c-myc exon is broken in the J558S α 4 and MPC-11myc3' clones (16,22). Only relevant restriction sites are indicated. NIARD and H2.4 indicate the locations of subcloned unique sequence probes specific for c-myc and MPC-11myc target respectively.

Figure 2

Southern transfer of BamHI digested mouse-hamster somatic cell hybrids, hamster and BALB/c DNAs hybridized to the MPC-11myc3' target probe, H2.4. A 6.2kb fragment corresponding to the H2.4 sequence in BALB/c DNA is only observed in MAE28, a mouse-hamster hybrid which uniquely contains mouse chromosome 12. Hybridization was performed essentially as described (21) but in the absence of dextran sulphate. The filter was subsequently washed at high stringency in 0.1XSSC, 0.1% SDS for 2 hrs. at 65°C prior to autoradiography.

RESULTS

From our previous work, we knew that the rearranged myc gene in MPC-11 was contained in a 16.5kb EcoRI fragment. Using NIARD (i.e. c-myc) specific probe, p α 25BH3.4 (2,13,16), we screened a partial MPC-11 library consisting of EcoRI fragments of 15.5 to 18.5kb cloned in Charon 4A.

As shown in Figure 1, the MPC-11 rearranged myc gene (MPC-11 myc3') is similar to the rearranged myc clones from J558 (and other myelomas where S_{α} is

Figure 3

Southern blot of EcoRI digested Igh recombinant and inbred mouse strains hybridized to the MPC-11myc3' H2.4 target sequence mapping H2.4 distal to IghD. Hybridization was performed as described with 10% dextran sulphate (21). The final filter wash was performed in 0.1% SDS for 30 minutes at 65 C. The intensity of hybridization of the 7.7 and 6.8kb bands was considerably diminished with higher stringency washes as performed in Figures 2 and 5. See Table 1 for crossover points.

the target) (1-4). Restriction endonuclease mapping and nucleotide sequence analysis has confirmed that the J558 and MPC-11 rearrangements break the c-myc gene in a 5' non-coding exon, the MPC-11 break occurring 219 base pairs 3' of the J558 break (16,22 and Figure 1). At the extreme 5' end of MPC-11myc3' C_α and S_α are replaced by an unknown segment of DNA. In order to determine the origin and nature of this unknown 5' flanking DNA, we prepared a non-repetitive sequence probe to be used in genomic Southern blots. By probing a HindIII digest of MPC-11myc3' with total nick translated BALB/c DNA, we were able to show (by lack of detectable hybridization) that the 2.4 kb HindIII fragment at the 5' end of the clone would make a suitable probe. This fragment was subcloned into the HindIII site of pBR322. The subclone is

Table I
Myc H2.4 Maps Distal to Igh-V and Igh-D

Strains	Igh-V		Igh-D	Igh-C	Myc H2.4
B6	b		b	b	b
BALB/c	a		a	a	a
C.B-17	b		b	b	b
BAB/14	a	X	b	b	b
C.B/R3	b	X	a	a	a
C.B/R4	b	X	a	a	a
C.B/R5	a		a	X	b
C.B/R6	a	X	b	b	b
C.B/R7	a	X	b	b	b

- 1 Igh recombinant strains were derived from the initial cross of BALB/c X C57B1. C.B-17, BAB/14 and C.B-8Kn are BALB/c .C57B1-Igh allotype congenic strains. The latter two were found to have a recombinant Igh haplotype while C.B-17 does not. The C.B/R strains were derived by crossing BALB/c and C.B-17 and selecting for genetic crossovers in the Igh region. (Brodeur and Riblet, unpublished results).
- 2 Igh-V and Igh-D haplotypes were determined from Southern blots performed with V and D probes, while Igh-C was analyzed by serology. The alleles at each locus are listed and crossover points indicated as X (Brodeur and Riblet, unpublished results).

referred to as MPC-11myc3'H2.4 or H2.4.

The first step in determining the origin of the c-myc target in MPC-11 was to localize H2.4 to a specific chromosome. The obvious candidates were 12 (heavy chain genes), 6 (kappa light chain genes) and 15 (chromosome containing c-myc), thus covering T(12;15) and T(6;15) translocations which have been observed cytogenetically and intrachromosomal rearrangements, respectively. Using mouse-Chinese hamster somatic cell hybrids containing either mouse chromosome 12 + X (MAE28), 14 + 15 (ECm4e), or 6 + others (MACH 2A2) (23-26) and digesting with EcoRI, HindIII and BamHI we were able to localize H2.4 to chromosome 12 or X (BamHI data shown in Figure 2). Furthermore, H2.4 does not hybridize to mouse chromosomes 16 + X (MAE32) and is therefore located on chromosome 12.

We next localized H2.4 within chromosome 12 with respect to an obvious genetic marker, the Ig heavy chain genes. The immunoglobulin heavy chain constant region gene cluster (designated IgH-C) is one of several tightly linked loci known to reside at the distal end of mouse chromosome 12 (27-30). The genetic organization of the relevant loci for this study is as follows:

Table IIMyc H2.4 Site Maps Proximal to Tsu and Pre-1.

Strains	Igh-C	MycH2.4	Tsu	Pre-1
NZB	n	d	d	a
C58	a	a	-	o
NX8-13	a	a X	d	a
BALB/c	a	a	-	a
A/Icr	e	d	d	o
AXC-11	a	a X	d	o
AXC-12	a	a X	d	o
CXAXCB-1	e	d	d X	a
CXAXCB-2	a	a	- X	o
CXAXCB-3	e	d X	-	a
CXAXCB-4	e	d X	-	a
CXAXCB-5	e	d X	-	a
CXAXCB-6	a	a X	d	o
CXAXCB-7	a	a	- X	o
BAB/14	b	b	-	o
C.AL-9	d	d	d	a
C.B.AL-6	b	b X	d	a

- 1 In each group, the first two strains are the parents giving rise to the recombinant strains following. The construction and characterization of all strains are as follows: NX8-13 recombinant inbred line (28); AXC, CXAXCB and C.B.AL-6 Igh recombinant strains (27,29; Owen and Riblet, unpublished results).
- 2 Igh-C and Tsu haplotypes were determined by serology and Pre-1 by electrophoretic variation. The alleles at each locus are listed and recombination points are indicated by an X (27-29; Owen and Riblet, unpublished results.)

(centromere)...Igh-V...Igh-D...Igh-C...Tsu...Pre-1 (21,23-24,30). Igh-V and Igh-D refer to the immunoglobulin Variable and Diversity DNA segments (31-32). (We are aware that in humans there is evidence that the orientation of the Igh genes with respect to the centromere is opposite of this (30,33) but our arguments are not affected.) Tsu is a T cell alloantigen (30) and Pre-1 is prealbumin (34). This analysis was made possible due to a restriction endonuclease polymorphism exhibited by H2.4 homologous sequences in two different inbred mouse strains, C57Bl/6 (B6) and BALB/c (see Figure 3 for B6 and BALB/c EcoRI restriction patterns). Recombinant strain patterns (Figure 3) were used to map H2.4 relative to Igh loci. The experiment summarized in Table I was designed to localize H2.4 proximal or distal to the Igh-D elements. Using DNA from recombinant mouse strains having crossovers between

Figure 4

Southern blot EcoRI digested Igh recombinant and inbred mouse strains hybridized to the MPC-11myc3' H2.4 target sequence mapping H2.4 proximal to Tsu. Hybridization and washing conditions were as listed in Figure 3. See Table 2 for crossover points.

Igh-V and Igh-D, we were able to show that H2.4 consistently segregates with the Igh-D locus. However, in recombinant strain C.B/R5 which contains a crossover between Igh-D and C_H gene cluster, H2.4 segregates with the C_H genes. This maps H2.4 distal to Igh-D. To further localize H2.4 with respect to the Igh-C genes, we employed other groups of recombinant strains having crossovers between Igh-C and Tsu. Table II shows that H2.4 consistently segregates with Igh-C (restriction patterns shown in Figure 4), thus indicating that it is localized proximal of Tsu. These two experiments localize H2.4 between Igh-D and Tsu.

In light of these results, we compared a restriction endonuclease map of the MPC-11 myc target with a map of the entire C_H gene cluster (35). As shown in Figure 5, the MPC-11 myc target closely resembles a sequence found 5' of the $C_{\gamma 2a}$ gene. In confirmation of this observation a 5' flanking $C_{\gamma 2a}$ probe pS $\gamma 2a$ -1 was observed to strongly hybridize to the H2.4 sequence in MPC-11myc3' (data not shown). In Figure 5, we show that c-myc, S $\gamma 2a$ and H2.4 probes each detect a 16.5kb EcoRI fragment in MPC-11 DNA and S $\gamma 2a$ or H2.4

probes hybridize to identical bands in BALB/c DNA. Therefore, the two H2.4 hybridizing sequences present in various mouse strains in Figure 3 represent the homologous S_{γ2b} and S_{γ2a} regions which are known to display length variation in different mouse strains (35, Stanton and Marcu, unpublished results).

In addition to the 16.5kb myc rearrangements, S_{γ2a} and H2.4 probes also detect 6.8 and 5.9kb rearranged bands in MPC-11 (see Figure 5). The 6.8kb band corresponds to a rearranged γ2a gene which is present in MPC-11 cells (21, L.A. Eckhardt and B.K. Birshtein, personal communication). The 5.9kb band is unique to our particular sample of MPC-11 cells and is not observed in other independently maintained stocks of MPC-11 cultured cells (Eckhardt and Birshtein personal communication). The origin of this novel 5.9kb band remains unknown and may represent a secondary rearrangement of the 6.5kb band. The 16.5kb myc associated S_{γ2a} band is found in all stocks of MPC-11 tested and in MPC-11 switch variants (22, unpublished results). We also show in Figure 5 that an S_{γ2b} probe, pBR1.4 (21) does not detect this 16.5kb band in MPC-11. In a previous study, we had concluded that the MPC-11 myc rearrangement did not involve a C_H S region (2). This assumption was based in part on our inability to detect the NIARD rearrangement with S_{γ3} and S_{γ2b} probes which possess homology with S_{γ2a} (35,36). The reasons for this discrepancy are not clear but may reflect the high stringency of some genomic hybridizations and possibly the loss of S sequences upon c-myc translocation.

In light of this unexpected finding, we investigated the myc targets in other IgG producing plasmacytomas. As shown in Figure 6A, Southern hybridizations of EcoRI digested DNAs of PC3612, and PC3791 (two γ2b producing NZB tumors) display a rearranged myc sequence. The rearranged c-myc sequences in PC3701 and PC3612, were observed to co-migrate with sequences which hybridize to an S_{γ2b} probe (see Figure 6B). These results were

Figure 5

Identification of the MPC-11 myc target as the γ2a switch region. Southern blots of EcoRI digested BALB/c and MPC-11 DNAs were hybridized to NIARD, S_{γ2b}, S_{γ2a} and H2.4 probes as shown. A comparison of restriction maps of the MPC-11 myc3' target and the 5' flanking region of the γ2a heavy chain gene is also presented. The 6.8kb and 3.2kb MPC-11 specific bands which hybridize to an S_{γ2b} probe, pBR1.4 (21), correspond to two rearranged S_{γ2b} sequences (21). The identities of other specific bands are indicated and discussed in the text. Hybridizations and filter washes were performed as described for Figure 2.

Figure 6

Southern blots of EcoRI restricted NZB liver, PC3612 and PC3701 DNAs hybridized to NIARD (panel A) and S γ _{2b} (panel B) probes. Arrows in panel A correspond to rearranged myc sequences which are also observed to hybridize to an S γ _{2b} probe in panel B. Rearranged S γ _{2b} specific bands of 3.55 and 4.8kb in PC3612 and PC3701 are denoted F γ _{2b} and represent the functionally rearranged γ _{2b} genes in these two IgG2b producing plasmacytomas.

confirmed by HindIII digestion (data not shown). Similar hybridizations performed under identical stringency with S γ ₃, S γ ₁ and S γ _{2a} probes yielded no c-myc co-migrating bands (data not shown). These results confirm that the c-myc targets in PC3612 and PC3701 are the S γ _{2b} region.

DISCUSSION

The S γ -myc rearrangements described here as well as the large number of S α -myc rearrangements (37) strongly implicate C_H switch regions in most if not all myc associated translocations in BALB/c and NZB plasmacytomas. Although translocation events involving the kappa and lambda loci have been postulated based on cytogenetic evidence from some tumors, such myc associated

translocations have not been described. Thus far myc targets have been found to exclusively reside with the unexpressed C_H locus (1-4,37, this report). An analogous chromosome translocation, T(8;14), in human Burkitt lymphomas has been shown to generate aberrant myc-S $_{\mu}$ rearrangements (12,13,15) and recently a human c-myc-S $_{\gamma 1}$ recombination has also been identified (38). The preference for S $_{\alpha}$ targets in the myc rearrangements of most BALB/c plasmacytomas may be a reflection of the large number of BALB/c IgA producers. The presence of S $_{\gamma}$ myc targets in some IgG producers could possibly imply an interesting relationship between the expressed C_H class and the S region myc target. However, of 12 gamma producing plasmacytomas examined, four had no c-myc rearrangements, three recombined with S $_{\gamma 2b}$ (this report), four recombined with S $_{\alpha}$ and one has apparently recombined with non-switch region sequence (E.F. Remmers, L.J. Harris and K.B. Marcu, unpublished results). Also two BALB/c IgG producers were found to contain myc-S rearrangements (37). Therefore an absolute correlation between expressed C_H class and the S region myc target cannot be made. Thus far c-myc genes have always been observed to recombine on the excluded Igh chromosome either with the same switch region as the expressed C_H allele or at a downstream S region.

In spite of a strong preference for S region targets, c-myc exon and intron sequences involved in this recombination lack any obvious homology with S region repetitive sequences (2-4,16,37). Interestingly, the majority of functional C_H gene switches' defined at the molecular level in murine plasmacytomas are not generated by direct recombination between homologous S region sequences (39). Similarities between the c-myc locus and C_H S regions may conceivably reside in a higher order secondary structure which is shared between the c-myc translocation region and S sequences. The assignment and molecular analysis of myc targets in other IgG producing plasmacytomas will be required to assess the significance of any preference for specific S region like sequences in this rearrangement.

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