

Previously Hidden Chromosome Aberrations in T(12;15)-positive BALB/c Plasmacytomas Uncovered by Multicolor Spectral Karyotyping

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ABSTRACT

The majority of BALB/c mouse plasmacytomas harbor a balanced T(12;15) chromosomal translocation deregulating the expression of the proto-oncogene *c-myc*. Recent evidence suggests that the T(12;15) is an initiating tumorigenic mutation that occurs in early plasmacytoma precursor cells. However, the possible contribution of additional chromosomal aberrations to the progression of plasmacytoma development has been largely ignored. Here we use multicolor spectral karyotyping (SKY) to evaluate 10 established BALB/c plasmacytomas in which the T(12;15) had been previously detected by G banding. SKY readily confirmed the presence of this translocation in all of these tumors and in three plasmacytomas newly identified secondary cytogenetic changes of the *c-myc*-deregulating chromosome (Chr) T(12;15). In addition, numerous previously unknown aberrations were found to be scattered throughout the genome, which was interpreted to reflect the general genomic instability of plasmacytomas. Instability of this sort was not uniform, however, because only half of the tumors were heavily rearranged. Seven apparent hot spots of chromosomal rearrangements (40% incidence) were identified and mapped to Chrs 1B, 1G-H, 2G-H1, 4C7-D2, 12D, 14C-D2, and XE-F1. Two of these regions, Chr 1B and Chr 4C7-D2, are suspected to harbor plasmacytoma susceptibility loci; *Pctr*¹ and *Pctr*² on Chr 4C7-D2 and as yet unnamed loci on Chr 1B. These results suggest that secondary chromosomal rearrangements contribute to plasmacytoma progression in BALB/c mice. To evaluate the biological significance of these rearrangements, SKY will be used in follow-up experiments to search for the presence of recurrent and/or consistent secondary cytogenetic aberrations in primary BALB/c plasmacytomas.

INTRODUCTION

The cytogenetic hallmark of virtually all pristane-induced plasmacytomas developing in genetically susceptible BALB/c mice is a balanced chromosomal translocation that results in the deregulated, constitutive expression of the proto-oncogene *c-myc* (1). The predominant translocation is the T(12;15), which juxtaposes *c-myc* residing on Chr² 15 to genes of the *Igh* locus on Chr 12 (2). Less frequently observed are the reciprocal translocations, T(6;15) and T(15;16), recombining a locus that is located 220 kbp 3' of *c-myc*, *pvt-1*, with the *Igκ* light-chain locus on Chr 6 or the *Igλ* light-chain locus (*Igλ*) on Chr 16 (3-6). Thus, all three functional immunoglobulin gene clusters are used in mouse plasma cell tumors in distinct *c-myc*-deregulating transchromosomal rearrangements, and all three of them can be detected by conventional karyotyping with Giemsa-banded chromosomes. Interestingly, the

spectrum of *c-myc*-deregulating translocations that is observed in mouse plasmacytomas is very closely reproduced in Burkitt's lymphoma, a human B-cell lymphoma in which *c-myc* residing on Chr 8 is activated by translocations T(8;14), T(8;22), and T(2;8), recombining *c-myc* with genes of the *Igh*, *Igκ*, and *Igλ* loci, respectively.

Formal proof of the presence of any of the *c-myc*-deregulating chromosomal translocations can only be established by cytogenetic techniques. However, we have recently developed a number of direct PCR methods to confidently detect the T(12;15) in plasmacytoma cells and their precursors, which has allowed us to overcome some of the technical limitations of cytogenetics with respect to the number, location, and type of B cells that can be scrutinized for translocations (7, 8). One important result that was obtained by PCR analysis was the finding that recombinations between *c-myc* and *Igh* (typical of T(12;15)) can consistently be detected in B lymphocytes recovered 7 and 30 days after initiation of tumor induction. However, plasmacytomas need about 8 months to develop (mean latency period is approximately 220 days). This situation suggests that the T(12;15) is an initiating oncogenic mutation that takes place in early tumor precursor cells (9, 10).

Secondary genetic changes have been postulated to occur in the course of plasmacytoma development, and it seems reasonable to hypothesize that they might include chromosomal aberrations occurring subsequent to the *c-myc*-activating translocations. However, such abnormalities have not been detected thus far in BALB/c plasmacytomas. In fact, the only recurrent secondary cytogenetic change that has been found by conventional karyotype analysis is trisomy 11 in a subset of virally induced plasmacytomas (6, 11). The main reason for the failure to detect additional chromosomal aberrations in pristane-induced plasmacytomas may be a technical one, namely the inherent difficulty and insensitivity of G banding in the mouse. Mouse cells contain 20 acrocentric chromosomes that are similar in size and, therefore, much harder to identify unambiguously than their human counterparts. To overcome the limitations of conventional cytogenetic analysis, we applied a recently developed molecular cytogenetic method, multicolor chromosome painting, or SKY, which allows us to visualize each chromosome in a different color (12, 13). All mouse chromosomes, and even large chromosome fragments, can be objectively identified by SKY, eliminating many of the uncertainties and ambiguities of conventional karyotyping in mouse tumors.

Here, SKY is used to assess the entire chromosome complement of 10 established plasmacytoma cell lines that had been previously karyotyped by G banding. The analysis revealed not only a multitude of unique, inconsistent aberrations (most of which were seen for the first time) but also seven recurring hot spot regions of illegitimate genetic recombinations. Two of these regions are suspected to harbor plasmacytoma susceptibility loci: *Pctr*¹ and *Pctr*² on Chr 4C7-D2 and additional susceptibility gene(s) on Chr 1B that have not yet been fine mapped.

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² The abbreviations used are: Chr, chromosome; HOPC, 7-*n*-hexyloctadecane-induced plasmacytoma; MPC, Merwin plasmacytoma; MOPC, mineral oil-induced plasmacytoma; RPC, Robertson plasmacytoma; SKY, spectral karyotyping; TEPC, tetramethylpentadecane-induced plasmacytoma; *Igh*, immunoglobulin heavy chain; DAPI, 4',6-diamidino-2-phenylindole; RGB, red, green, and blue.

Table 1 BALB/c plasmacytomas analyzed by SKY

Plasmacytoma ^a	Induction ^b	Chr ^c	Igh ^d	c-myc ^e	Year ^f	Source ^g	Ref.
HOPC 1 ^h	Alkane	74	$\gamma 2\alpha$	Ig α	1969	TIB-13	27, 28
MOPC 104E ⁱ	Mineral oil	98	μ	Ig α	1962	PerImmune	28-30
MPC 11	Plastic chamber	63	$\gamma 2b$	Ig α	1970	15-TIB	28, 31, 32
TEPC 1033	Pristane	81	δ	Ig $\gamma 2a$	1978	PerImmune	33
TEPC 1165 ^j	Pristane	66	α	Ig α	1981	PerImmune	34
TEPC 2027 ^k	Pristane	75	$\gamma 1$	Ig $\gamma 2b$	1983	PerImmune	34
RPC 5 ^l	Adjuvant	61	$\gamma 2a$	NI	1959	TIB-12	35, 36
SP2/0 ^m	Mineral oil	68	None	Ig $\gamma 2b$	1978	CRL-8287	37
S 107 ⁿ	Mineral oil	67	α	Ig α	1967	PerImmune	28
XRPC 24 ^o	Pristane	68	α	Ig α	1972	PerImmune	38

^a Plasmacytoma is a malignant lymphoma corresponding to terminally differentiated B lymphocytes, plasma cells. For current reviews on plasmacytoma development in BALB/c mice, including many details concerning the tumors included in this study, see Refs. 1 and 39.

^b The tumor induction regimen consisted in all cases except MPC 11 of three i.p. injections of 0.5 ml of an oily tumor-promoting compound that were spaced 2 months apart. Different agents, such as mineral oils, pristane, or other alkanes, were used to induce plasmacytomas. Mineral oils, such as Bayol F, are complex aliphatic mixtures (paraffin oils) that contain pristane (40). Pristane (2,6,10,14-tetramethylpentadecane) is the best-studied and most widely used plasma cell tumor-inducing alkane (41-45). HOPC 1 was induced by 7-n-hexyloctadecane, a cyclic alkane with structural similarities to pristane. RPC5, which is also known as ADJPC 5, was induced by i.p. administration of Lieberman's staphylococcal adjuvant (46), the main plasmacytomagenic component of which is mineral oil (40). MPC 11 developed in the reactive tissue surrounding a Millipore diffusion chamber that was implanted in the peritoneal cavity of a BALB/c mouse (47).

^c Modal chromosome number determined with DAPI- and/or Giemsa-stained metaphase spreads. Ten to 20 karyotyped plates were analyzed per tumor. Most plasmacytomas were found to be in the hyperdiploid to subtetraploid range, except for MOPC 104E and TEPC 1033, which were hypertetraploid.

^d Class of Igh that is produced and secreted by malignant plasma cells.

^e Igh locus that was found to have undergone recombination with c-myc on the c-myc-deregulating product of the reciprocal chromosomal translocation T(12;15), Chr 12⁺. The breakpoint regions of most tumors were recently determined by direct PCR (7, 8, 48).³ MOPC 104E, MPC 11, and S 107 were analyzed by molecular cloning (28, 29, 32). NI, not investigated.

^f Year in which the tumor was induced (49) or described in the literature for the first time.

^g Plasmacytomas were obtained as cell lines from PerImmune, Inc. (Rockville, MD) or from American Type Culture Collection (Rockville, MD), in which case the catalog number is given.

^h Resistant to cortisol.

ⁱ MOPC 104E harbors a deletion of Igh α on one copy of Chr T(12;15), producing the unusual rearrangement between the 3' flank of C α with c-myc (29). The secreted immunoglobulin is an antibody of known specificity to $\alpha 1 \rightarrow 3$ dextran (50).

^j TEPC 1165 harbors a splicing mutation in c-myc on Chr T(12;15), which results in an exceptionally stable c-myc message (34). Cells are dependent on IL-6 in order to grow *in vitro* (14) and *in vivo* (51).

^k TEPC 2027 is dependent on the addition of IL-6 for *in vitro* growth (14).

^l The subline of RPC 5 used here, RPC 5.2, is resistant to 8-aza-guanine (36).

^m The subline of SP2/0 chosen here, SP2/0-Ag14, has been derived from the myeloma cell line P3X63Ag8, a 8-azaguanine-resistant variant of MOPC 21, which was originally induced in 1960 (49). The tumor harbors a c-mos proto-oncogene that has been activated by insertion of an intracisternal A particle (IAP) (52). In SP2/0 cells, a T(6;10) has been described recombining the proto-oncogene *mdm2* on Chr 10 with Ig κ on Chr 6 (23).

ⁿ The secreted immunoglobulin is an antibody of known specificity to phosphorylcholine (50).

^o The tumor was induced in a BALB/c mouse that had been treated with pristane and ionizing radiation. Because of the irradiation, the tumor was named X-ray-induced plasmacytoma (XRPC), even though irradiating the mouse was probably not critical for plasmacytoma development. The secreted immunoglobulin is an antibody of known specificity to $\beta 1 \rightarrow 6$ D-galactan (50). The tumor harbors a c-mos proto-oncogene that has been activated by insertion of an intracisternal A particle (53).

MATERIALS AND METHODS

BALB/c Plasmacytomas. Ten established plasmacytomas were included in this study. Their salient features are summarized in Table 1. The tumors were induced in different laboratories in BALB/c mice that had been subjected to one of several plasmacytoma induction regimens. The most commonly used protocol used three 0.5-ml injections of mineral oil or pristane spaced 2 months apart. Over the years, plasmacytomas have been maintained by *in vivo* passaging into recipient BALB/c mice, and continuous cell lines have been established as well. Some of these long-term cell lines are available at our conventional mouse facility (PerImmune, Inc., Rockville, MD) under NCI contract N01-BC-21075. Plasmacytomas were grown in suspension culture in RPMI 1640 containing 10% FCS, 200 mM L-glutamine, and 50 mM 2-mercaptoethanol at 37°C in a humidified atmosphere containing 5% CO₂ in air. Two tumors, TEPC 1165 and TEPC 2027, were strictly dependent on the presence of IL-6 (14), which was added at 100 plasmacytoma units/ml of cell culture medium.

SKY. SKY was performed exactly as described elsewhere (12, 13). Briefly, mouse metaphase chromosomes were prepared from plasmacytoma cell lines according to standard procedures (Fig. 1A). Chromosome-specific painting probes were generated from flow-sorted chromosomes. DNA prepared from sorted chromosomes was amplified by degenerate oligonucleotide-primed PCR incorporating haptenized or fluorochrome-conjugated nucleotides (15). Labeled probes were hybridized *in situ* for 2 days in the presence of a large excess of unlabeled Cot-1 fraction of mouse genomic DNA (Bethesda Research Laboratories). Biotin- and digoxigenin-haptenized nucleotides of chromosome-specific probes were detected with avidin-conjugated Cy5 and antibody-conjugated Cy5.5 (using mouse antidigoxigenin antibody sandwiched by goat antimouse antiserum), respectively. Chromosomes were counterstained

with DAPI. Spectral analysis of chromosomes was carried out on an inverted microscope (Leica DMIRBE) that was equipped with a SD200 spectral cube (Applied Spectral Imaging) and a custom-designed filter cube (SKY1, Chroma Technology, Brattleboro, VT) that allows for the simultaneous excitation of all dyes and the measurement of their emission spectra. The spectral measurement of the hybridization was visualized by assigning a RGB look-up table to specific sections of the emission spectrum (Fig. 1B). For instance, the X chromosome, labeled with Spectrum Green, appears blue, Chr 16 (Cy3) appears green, and Chr 2 (Cy5 and Cy5.5) appears red (Fig. 1B). The RGB display allows the assessment of important parameters of the hybridization [e.g., intensity and homogeneity (Fig. 1B)]. On the basis of the measurement of discrete emission spectra at all pixels of the image, the hybridization colors are then converted by applying a spectral classification algorithm that results in the assignment of a discrete color to all pixels with identical spectra (Fig. 1C). The spectral classification is the basis for chromosome identification and SKY (Fig. 1C; Ref. 16).

RESULTS AND DISCUSSION

T(12;15). The presence of the expected reciprocal chromosomal translocation, T(12;15), was readily confirmed by SKY in all 10 tumors included in this study. A typical example of this translocation, as detected in TEPC 1165, is shown in Fig. 1B, *inset*, illustrating the rearrangement of c-myc, which is located at band D2-D3 of Chr 15, with some portion of the Igh gene cluster, which resides at a position 58 cM from the centromere on band F1 of the 61-cM-long Chr 12. The balanced genetic exchange produced a large Chr T(12;15), containing most of Chr 12, and a small Chr T(15;12), harboring a telomeric portion of Chr 12 fused to the broken Chr 15. The two derivative chromosomes are clearly visualized by chromosome painting, which,

³ A. L. Kovalchuk, unpublished results.

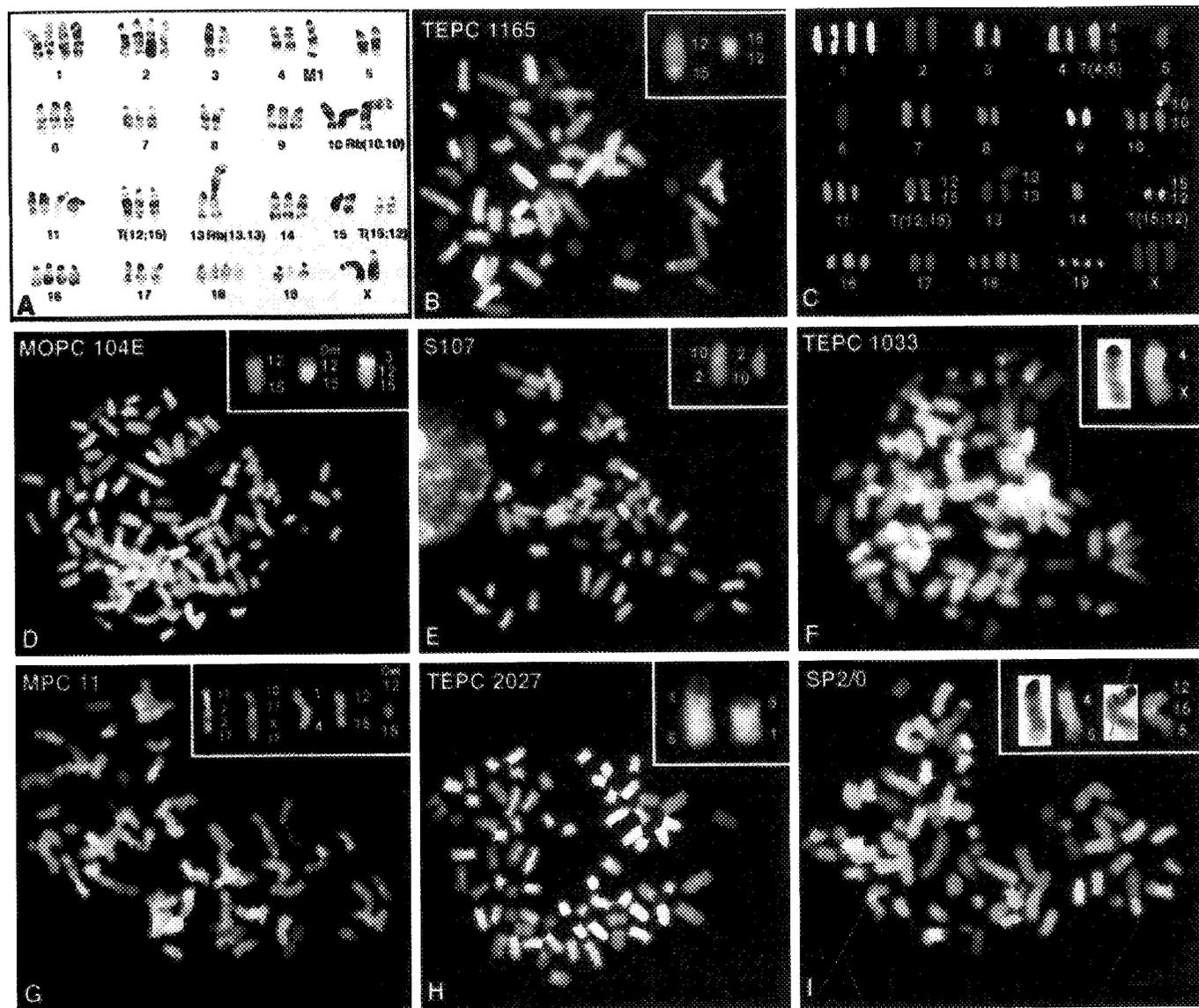


Fig. 1. Chromosomal aberrations in long-term BALB/c plasmacytoma cell lines as observed by SKY. One representative metaphase spread of seven plasmacytomas is shown. Three tumors included in this study are not illustrated, namely HOPC 1, RPC 5, and XRPC 24 [which has already been depicted elsewhere (12)]. A comprehensive cytogenetic analysis of TEPC 1165 is shown at the top. A, G-banding analysis in which several aberrant chromosomes (labeled) and one marker chromosome (M1) were found; B, SKY of metaphase chromosomes (obtained from another spread) in which the chromosome spectra were visualized in display colors by assigning a RGB look-up table to different regions of the spectrum (see "Materials and Methods"); C, spectral karyotype after spectrum-based classification in which chromosomes were assigned a pseudocolor (classification color) according to the measured spectrum (see "Materials and Methods"). The two Robertsonian translocations, the reciprocal T(12;15), and the marker chromosome could clearly be identified. The marker chromosome, which was suspected to be a Chr 4* by G banding, was identified as a T(4;5) by SKY. Note that a piece of Chr 1 is contained in the Rb(10;10) classification image (C), which was caused by an overlap of the two chromosomes in the metaphase spread used for the display image (B, arrow). D-I, SKY display images of karyotypes from six cultured plasmacytomas. From each tumor, aberrant chromosomes were selected for illustration (see insets). Images of G-banded or DAPI-stained chromosomes are included in some cases for reference. Some of the hybrid chromosomes shown here in SKY display colors can be compared to their images in SKY classification colors illustrated in Fig. 2: T(1;4) in MPC 11; T(4;5) in SP2/0; T(Del12;15) in MOPC 104E; and T(4;X) in TEPC 1033.

according to the fluorescence labeling schema of mouse chromosomes chosen here, displays Chr 12 in pink (using a combination of Spectrum Green- and Cy5-labeled probe) and Chr 15 in blue (combining Spectrum Green- and Cy3-labeled probe; see Fig. 1B, inset). Note that the small pink portion on Chr T(15;12) represents as little as 3 cM (~7.5 Mbp) of Chr 12, and its visibility attests to the sensitivity of SKY to identify chromosomes with a very small chimeric portion.

Secondary Rearrangements of Chr T(12;15). Another example of a typical Chr T(12;15) is illustrated in Fig. 1G, inset (second chromosome from the right). Interestingly, in the same plasmacytoma, MPC 11, an additional copy of Chr T(12;15) was identified, which appears to harbor a large deletion of the Chr 12-derived portion of the hybrid chromosome, Chr T(12;15). In contrast, the Chr 15-derived

portion is apparently unchanged (far right chromosome in Fig. 1G, inset). A similar example of a plasmacytoma with coexisting copies of typical and abnormal Chr T(12;15) was encountered in MOPC 104E, in which a normal-sized Chr T(12;15), a second chromosome with a large deletion in the Chr 12-derived part, T(Del12;15), and a third chromosome with a secondary recombination between the Chr 12 and Chr 3, T(3;12;15), were found to be present (see first, second, and third chromosomes from the left in Fig. 1D, inset, respectively). Tumor SP2/0 is another case in which Chr T(12;15) underwent a secondary translocation to a third chromosome, generating a T(12;15;5) (Fig. 1I, inset, right). As suggested earlier (17), a tetraploidization event subsequent to the T(12;15) is the most plausible explanation for the presence of both normal and aberrant versions of Chr

T(12;15) in plasmacytoma cells. Tetraploidization occurs, according to this schema, as a progression event resulting in the duplication of both translocated chromosomes, Chrs T(12;15) and T(15;12). It appears that one intact copy of Chr T(12;15) is consistently retained in plasmacytomas, whereas the second copy is subject to additional changes (e.g., deletions, as observed in MPC 11 and MOPC 104E, or transchromosomal rearrangements, as seen in MOPC 104E and SP2/0). It is currently unclear whether the secondary alterations of Chr T(12;15) suggest a general genetic instability of Chr 12 in BALB/c mice or, possibly, a more specific form of instability triggered by a chromosome scanning mechanism that senses the misplacement of the *Igh* gene cluster in the abnormal genomic context of Chr 15.

Mandatory Presence of Deregulated *c-myc*. If the deregulated expression of *c-myc* message and protein is required for both the development of plasmacytomas and the maintenance of the malignant phenotype during serial transplantation, the consistent presence of Chr T(12;15) harboring the deregulated *c-myc* gene should be expected. This prediction appears to be confirmed in all tumors of this series, because Chr T(12;15) was always present. The finding also suggests that transcriptionally active *c-myc* is not only important for plasmacytomas *in vivo* but is equally indispensable in plasmacytoma cell lines that have been maintained over many years *in vitro*. An additional question is raised by the presence of both normal and aberrant copies of Chr T(12;15) in three plasmacytomas, MOPC 104E, MPC 11, and SP2/0: from which Chr T(12;15) is *c-myc* actually transcribed? No molecular studies have been conducted thus far to address that issue in these three tumors. However, evidence obtained from other plasmacytomas (18–22) suggests that *c-myc* can be transcribed from chromosomes that have undergone complex rearrangements. Well-studied examples of plasmacytomas harboring an aberrant Chr T(12;15) with an atypical *c-myc*-deregulating genetic recombination include PC 7183 (18), ABPC 45 (19, 20), ABPC 60 (21), and DCPC 21 (22). Additional studies are warranted to compare the levels of *c-myc* transcripts expressed from conventional and secondarily rearranged Chrs T(12;15) and to elucidate the gene dosage effects likely to exist in a situation in which two, three, or four copies of Chr T(12;15) are present in a single tumor cell (see Table 2 for number of copies of Chr T(12;15) in MOPC 104E and XRPC 24).

Genomic Instability. A multitude of chromosomal aberrations were detected by SKY in addition to the omnipresent T(12;15). The changes included reciprocal, nonreciprocal, and Robertsonian translocations, deletions, insertions, dicentrics, as well as complex rearrangements (see Table 2 for a complete compilation of aberrations). Some types of chromosomal abnormalities, most importantly inversions, were not observed because of the inability of SKY to detect them. The extent to which inversions contribute to chromosomal rearrangements in BALB/c plasmacytomas must therefore be addressed by other methods. The analysis of the data presented in Table 2 reveals that many cytogenetic abnormalities were unique to individual plasmacytomas, most likely reflecting general genomic instability associated with repeated passages *in vivo* or *in vitro*. Nevertheless, some unique aberrations may still yield important information about candidate genes involved in plasmacytoma progression. For example, the reciprocal translocation T(6;10) was detected only once, namely in plasmacytoma SP2/0, suggesting general genetic instability. However, the molecular analysis of this translocation, which recombined *Igκ* with the mouse double minute gene *mdm-2*, a suspected inhibitor of p53 function, revealed the functional significance of the unique abnormality (23). Another example of a unique yet potentially significant translocation was found in a subclone of TEPC 2027, in which a reciprocal exchange between Chrs 1 and 5 was observed [see both derivative chromosomes, T(1;5) and T(5;1), in Fig. 1H, *inset*]. This particular subclone has acquired independence from

added IL-6, and we hypothesize that the translocation plays a critical role in that phenotype. This theory is supported by the location of the IL-6 gene on Chr 5 in the region in which the translocation occurred (band 5B, probably 5B3) and by the fact that the T(1;5) appears to be specific for the IL-6-independent subclone because it was not found in the IL-6-dependent precursor clone.

Not only do particular plasmacytomas have various characteristics potentially related to their specific genetic aberrations, but their overall degree of genetic instability, as measured by the number of aberrations that can be enumerated in a given plasmacytoma, appears to differ over a wide range as well. Half of the plasmacytomas studied here exhibited a relatively large number of distinct cytogenetic aberrations: 28 in SP2/0; 22 in MPC 11; 20 in RPC 5; 19 in M104E; and 10 in TEPC 2027. In contrast, the remaining tumors contained a much smaller number of visible rearrangements: 7 in S107; 5 in T1165 and XRPC 24; and just 3 in T1033 (see Table 2). The reason for the apparent difference between highly and minimally rearranged BALB/c plasmacytomas is not known, and no obvious correlation between any of the known features of plasmacytomas (see Table 1) and the severity of genetic instability can be established at this point. Thus, BALB/c plasmacytomas are not homogeneous with respect to overall genomic instability, and some long-term plasmacytoma cell lines present, surprisingly, a nearly normal karyotype.

Marker Chromosomes. A clear benefit of SKY is its ability to identify previously unidentifiable marker chromosomes. Some examples of such marker chromosomes usually generated by complex rearrangements involving three or four different chromosomes are illustrated in Fig. 1. Two marker chromosomes were identified in MPC 11 as T(10;17;X;17) and T(11;7;2;17). The composite images of these chromosomes are shown in Fig. 1G, *inset*. Furthermore, in RPC 5, a three-way translocation, T(2;3;X), was found (not shown). In other plasmacytomas, the nature of translocations previously suspected by G banding but not proven⁴ could be confirmed by SKY. Noteworthy examples include Chrs T(10;2) and T(2;10) in S 107, which are illustrated in Fig. 1E, *inset*; the T(9;19) that was also observed in tumor S 107 (not shown); and the T(1;2) in plasmacytoma SP2/0 (Fig. 2A, *far left*). Many additional examples of small aberrant chromosomes that would have been very difficult to identify by G banding could be mentioned here [e.g., Chrs T(1;2) in HOPC 1 (Fig. 2B), T(1;5) in MOPC 104E (Fig. 2B), T(14;11) in SP2/0 (Fig. 2F), and T(14;6) in MPC 11 (Fig. 2F)]. The G-banded Chr T(14;3) in MOPC 104E (Fig. 2F) and T(14;6) in MPC 11 (Fig. 2F) would have been classified as normal without SKY.

Hot Spot Regions. Analysis of the total sample of aberrations detected in 10 plasmacytoma cell lines points to hot spot regions of rearrangements, which were mapped to Chrs 1B, 1G–H, 2G–H1, 4C7–D2, 12D, 14C–D2, and XE–F1. Mapping was based on the comparison of SKY painted with G-banded chromosomes, allowing in most situations a confident assignment of the affected chromosomal bands or regions. In some instances, however, the precision of that determination was not fully satisfactory and must be augmented in future studies by combining SKY with high-resolution G banding, FISH and fiber FISH. Chromosomal breakpoint regions were defined as hot spots here when involved in 4 of 10 plasmacytomas. The probability of observing a recurrence rate as high as 40% by chance is extremely small. The individual rearrangements found in hot spot regions are illustrated in Fig. 2, in which ideograms of chromosomes harboring hot spots are shown, as well as images of hybrid chromosomes in spectral classification together with their G-banded or DAPI-stained counterparts. A detailed examination of Fig. 2 shows that

⁴ F. Wiener and A. E. Coleman, unpublished data.

Table 2. Summary of chromosomal aberrations in BALB/c plasmacytomas as detected by SKY

Plasmacytoma cell line ^a	Clone ^b	T (reciprocal) ^c	T (nonreciprocal) ^d	Robertsonian ^e	Deletions ^f	Insertions ^g	Coupled ^h	Additional aberrations ⁱ
HOPC 1	2	12;15 & 15;12 (clone a, 2, clone b, 3)	1;2, 5;1, 9;7 (clone a) 17;3 (clone b)		2 (clone a), 17 (clone a)			Dp(3) (clone b)
MOPC 104E	2	12;15 & 15;12 (clone a, 3; clone b, 4)	1;5 (clone a), 5;3, 5;9 6;3 (clone b), 6;18 (clone b), 11;14 (clone a) 14;3, 14;5		10	1;13	T(Del11;11) T(Del12;15) (clone b) T(Del14;3)	T(3;12;15) T(5;1;4;2)
MPC 11	1	12;15 (2) & 15;12	1;4, 1;5, 5;11 6;14, 9;8, 13;11 14;3, 14;6, 14;9 17;11;X;1	9;9 19;19			Is(1;9)Bnr T(6;9) T(Del12;15)	Del(Dic5) T(3;11;6) T(10;17;X;17) T(11;7;2;17) T(11;14;6)
RPC 5	2	12;15 (2) & 15;12	2;6, 3;X, 5;7 (clone a) 5;8, 5;18, 7;1 (clone b) 11;8, 12;10, 12;17 17;1, 17;19, 19;17 (clone b) X;2 (clone a)		X (clone a)		Rb(1;1)Bnr T(1;2;3) Rb(4;4)Bnr T(4;X;1)	T(17;19;1) (clone a) T(2;3;X) (clone b)
S 107	1	2;10 & 10;2 12;15 (2) & 15;12	5;1, 9;19					Dp(3)
Sp2/0	2	1;18 & 18;1 4;5 & 5;4 (clone a) 6;10 (clone b) & 10;6 (clone b)	1;2, 2;14, 5;1 7;2 (clone b), 7;9 (clone b), 9;18 (clone b) 11;3, 12;19 (clone a), 14;11		1, 2 (clone a) 4, 6	1;9 (clone a)	Rb(9;9)Bnr T(9;7)	T(12;15;5)
TEPC 1033	1	12;15 (2) & 15;12	4;X					
TEPC 1165	1	12;15 (2) & 15;12	4;5	10;10, 13;13				
TEPC 2027	2	1;5 (clone a) & 5;1 (clone a) 12;15 & 15;12 (2 (clone a), 1 (clone b))	3;14 (clone b), 4;2, 15;3 (clone b) 15;16 (clone a), 6;13 (clone b)					
XRPC 24	1	3;6 & 6;3 12;15 (3) & 15;12	1;X					

^a Compare Table 1.^b Number of separate clones that could clearly be distinguished. For cell lines in which two distinct clones were present, the aberrations specific for one particular clone, designated "a" or "b", are indicated; aberrations occurring in both clones are not further designated.^c Reciprocal translocations. Because of the significance of the T(12;15) for BALB/c plasmacytoma development, the number of Chrs T(12;15), including those that have undergone additional rearrangements such as deletions or translocations, is indicated in parentheses.^d Nonreciprocal translocations. Only one product of the interchromosomal exchange is present; the reciprocal product is deficient, most likely due to malsegregation in mitosis.^e Robertsonian translocations fuse two homologous or heterologous chromosomes at their centromeres.^f Deletions are defined here as cytologically visible, interstitial losses of chromosomal portions.^g Insertions are defined here as interstitial gains of chromosomal material that are cytologically visible. The chromosome donating the inserted portion is given first, followed by the recipient chromosome.^h Coupled complex aberrations involving two or more abnormalities. For example, Is(1;9)Bnr T(6;9) denotes an insertion of a portion of Chr 1 into Chr 9 which has also undergone translocation with Chr 6. Rb(1;1)Bnr T(1;2;3) designates a Robertsonian fusion between two copies of Chr 1, with one of them being rearranged with a translocated hybrid Chr 2;3. T(Del12;15) is regarded as one chromosome abnormality contained within another and designates a deletion in the Chr 12-derived part of translocated chromosome T(12;15).ⁱ Additional aberrations that are not listed in preceding columns; mainly three- or four-way translocations. Two duplications in Chr 3, Dp(3), and a deletion in a dicentric Chr 5, Del(Dic5), that was found in MPC 11 are also included.

those plasmacytomas identified as exhibiting high genomic instability, MOPC 104E, MPC 11, RPC 5, and SP2/0, contributed most of the rearrangements occurring in hot spot regions. Indeed, 22 of 28 aberrations in hot spot regions were found in these four plasmacytomas, raising the possibility that the recurrent rearrangements identified in this study are not common in BALB/c plasmacytomas but are confined to a subgroup of tumors characterized by a phenotype of high overall genomic instability.

Promiscuity at Hot Spots. The majority of chromosomal aberrations observed in hot spot regions appeared to be promiscuous with respect to the partner chromosomes of recombination. For example, the hot spot region on Chr 4C7-D2 was found to have undergone translocation with three different chromosomes in four different tumors: Chr 1 in MPC 11 (shown in the center of Fig. 1G, inset and also in Fig. 2A); Chr 5 in TEPC 1165 (Fig. 2D) and SP2/0 (Fig. 1I, inset and Fig. 2D, inset); and Chr X in TEPC 1033 (Fig. 1F, inset). Similarly, the hot spot region on Chr XE-F1 was used for translocations to three chromosomes in four plasmacytomas: Chr 1 in MPC 11

and XRPC 24; Chr 3 in RPC 5; and Chr 18 in SP2/0 (all shown in Fig. 2G). Further evaluation of Fig. 2 reveals the same level of promiscuity for the other five chromosomal hot spot regions on Chrs 1, 2, 12, and 14. Thus, it appears that recurrent rearrangements in BALB/c plasmacytomas usually take the form of promiscuous translocations, possibly indicating the presence of genes that might be recruited for plasmacytoma development by various translocation partners. This situation may be reminiscent of a promiscuous type of translocation in human leukemias disrupting the important target gene, *MLL*, and fusing it to various partner genes [e.g., *AF4/FEL*, *LTG9/AF9*, and *LTG19/ENL* (24, 25)].

Plasmacytoma Susceptibility Genes. In the absence of high-resolution mapping data, it may seem premature to contemplate the participation of particular genes residing in hot spot regions as candidate genes for plasmacytomagenesis. However, it is intriguing to note that two hot spot regions, Chrs 1B and 4C7-D2, have already been suspected to be the sites of plasmacytoma susceptibility genes. These genes were identified in plasmacytoma induction experiments

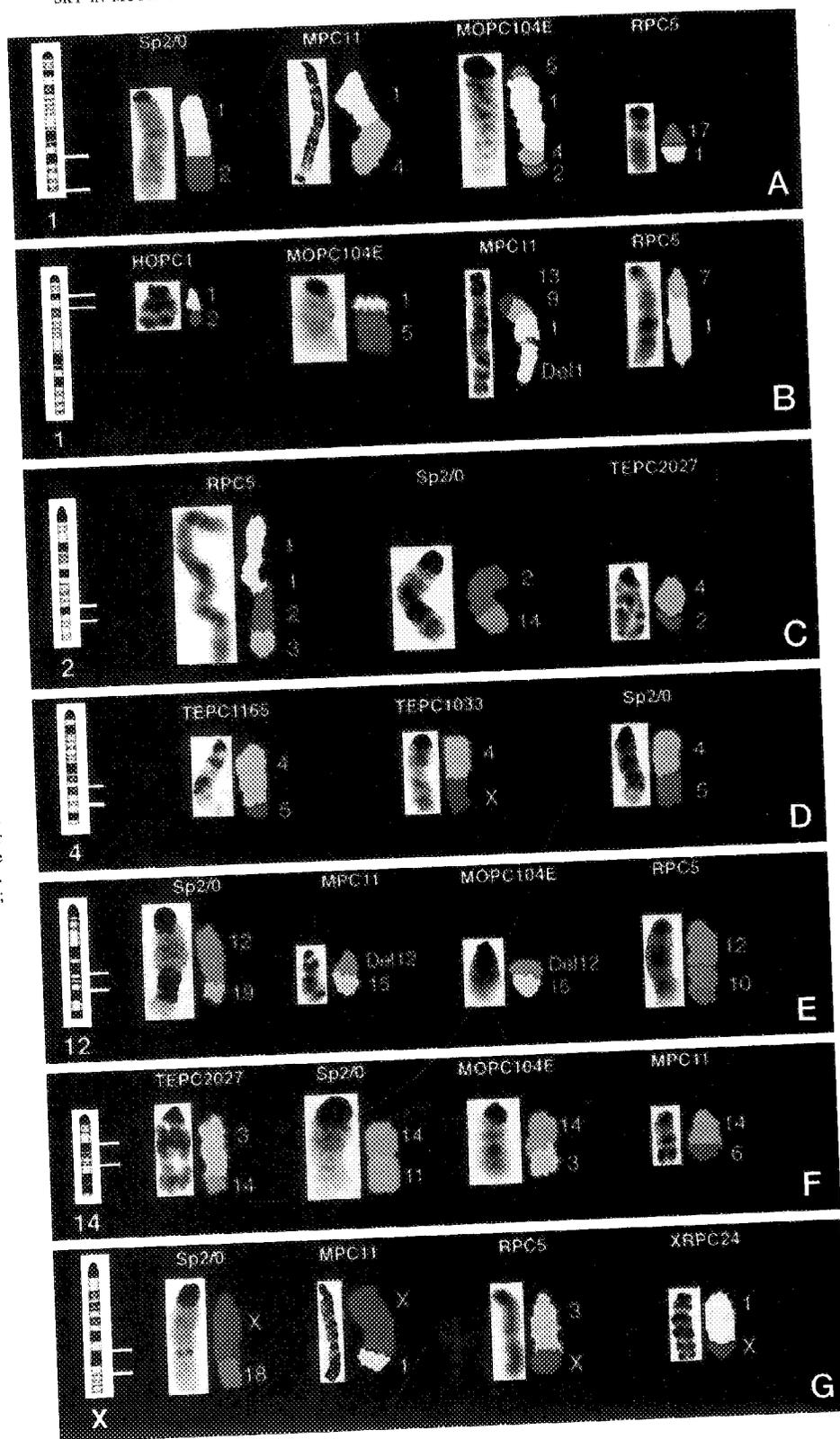


Fig. 2. Hot spots of chromosomal rearrangements in established cell lines of BALB/c plasmacytomas. Shown are ideograms of Chrs 1, 2, 4, 12, 14, and X, in which recurrent rearrangements (in 40% of tumors) were observed. Rearranged, hybrid chromosomes are shown in SKY classification colors. Giemsa-banded chromosomes (obtained from a different slide) or DAPI-stained chromosomes (obtained from the same slide) are included for comparison. Hot spot regions are indicated by white horizontal lines next to the ideograms and include 1B, 1G-H, 2G-H1, 4C7-D2, 12D, 14C-D2, and XE-F1. Chrs 2 and 4 are represented by three instead of four tumors. The reason is that the fourth example of a rearrangement involving these chromosomes, T(5;1;4;2) in MOPC 104E, is a complex translocation that is already shown in A. SKY-classified chromosomes may be compared to their counterparts in SKY display colors in the insets of Fig. 1. See Fig. 1D, inset, for T(Del12;15) in MOPC 104E; Fig. 1F, inset, for T(4;X) in TEPC 1033; Fig. 1G, inset, for T(Del12; 15) in MPC 11; and Fig. 1I, inset, for T(4;5) in SP2/0.

using a series of plasmacytoma-susceptible BALB/c strains that are congenic for portions of Chr 1 or Chr 4 derived from the plasmacytoma-resistant strain DBA/2N. Two DBA/2N-derived genes on the distal half of Chr 4, *Pctr*¹ and *Pctr*², have recently been identified, fine mapped to regions spanning *Ifa* and *D4Rck41* (*Pctr*¹) and *Tnfr-1* and *Pkc ζ* (*Pctr*²), and shown to confer resistance to plasmacytoma development (26). Other resistance genes are known to be present in a relatively large portion of Chr 1, spanning *Idh-1* and *Pep-3*, that has

not yet been fine mapped (26). The location of these loci raises the possibility that BALB/c plasmacytoma susceptibility genes residing on Chrs 4 and 1 may function as tumor progressor genes once they become activated by illegitimate genetic recombinations. It is known that the phenotype of plasmacytoma susceptibility, which is nearly exclusive to BALB/c mice, depends upon the cooperative effect of various strain-specific alleles that are distributed over several chromosomes (26). Thus, the hot spot regions of chromosomal aberrations

identified on Chrs 2, 12, 14, and X may indicate the location of additional susceptibility loci of plasmacytoma development in BALB/c mice.

In conclusion, SKY was applied to assess the karyotypic changes of 10 long-term T(12;15)-positive BALB/c plasmacytoma cell lines and proved to be a very effective method to identify a wide range of chromosomal aberrations, including marker chromosomes and complex genomic changes. The following new insights into the cytogenetics of BALB/c plasmacytomas were obtained. First, plasmacytomas seem to mandate the presence of at least one copy of Chr T(12;15) harboring the deregulated *c-myc* gene. In three cases, atypical, secondarily rearranged Chrs T(12;15) were also found. Second, BALB/c plasmacytomas appear to be heterogeneous with respect to overall genomic instability. Some tumors were heavily rearranged, whereas others, such as TEPC 1033, TEPC 1165, and XRPC 24, exhibited a nearly normal (hyperdiploid) karyotype. Third, some unique aberrations, although inconsistent, may still indicate important tumor progression events, as exemplified by the T(6;1) in plasmacytoma SP2/0 and the T(1;5) in TEPC 2027. Fourth, seven chromosomal regions were recurrently (40%) used in rearrangements, mainly in promiscuous transchromosomal exchanges, indicating the presence of hot spot regions of chromosomal aberrations in BALB/c plasmacytomas. Hot spot regions were mapped to Chrs 1B, 1G-H, 2G-H1, 4C7-D2, 12D, 14C-D2, and XE-F1. Significantly, two of these regions, Chrs 1B and 4C7-D2, have already been shown in genetic studies to harbor susceptibility genes of plasmacytoma development (26), supporting the notion that susceptibility genes may function as tumor progressor genes that become involved in plasmacytomagenesis through illegitimate genetic recombinations. In summary, SKY analysis revealed the presence of secondary genetic exchanges that appear to occur subsequent to T(12;15) in plasmacytoma cell lines. However, the significance of these cytogenetic aberrations for the development of plasmacytomas in BALB/c mice can only be fully appreciated once they have also been identified in primary plasmacytomas.

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