

Chromosomes 1 and 5 Harbor Plasmacytoma Progressor Genes in Mice

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Mouse spectral karyotyping (SKY) was employed to analyze 29 primary BALB/c plasmacytomas (PCTs) for the presence of chromosomal aberrations that took place subsequent to the *Myc*-activating T(12F1;15D2) or T(6C1;15D2) translocations, the initiating oncogenic mutations in plasmacytomagenesis. Recurrent amplifications of chromosome (Chr) 1 (48% prevalence) and promiscuous non-reciprocal translocations of Chr 5 (52% prevalence) suggested the existence of important PCT progressor genes on bands 1B/C and 5F. The additional occurrence of sporadic aberrations (93% prevalence) most likely reflected the general instability of the PCT genome. This instability, however, was not consistent, as two PCTs lacked secondary cytogenetic changes detectable by SKY. Our findings led us to conclude that BALB/c PCTs show a remarkably similar degree of cytogenetic heterogeneity to human multiple myeloma, despite being genetically defined (inbred mouse strain) and uniformly initiated (deregulation of *Myc*). *Genes Chromosomes Cancer* 29:70–74, 2000. Published 2000 Wiley-Liss, Inc.†

A considerable number of chromosomal aberrations have been identified in multiple myeloma (MM) (Cigudosa et al., 1998; Rao et al., 1998; Sawyer et al., 1998), and some have been associated with the activation of oncogenes or other genes involved in growth control (Chesi et al., 1997, 1998a,b; Iida et al., 1997). The resulting expectation, however, that the new findings can be translated expeditiously to an understanding of myelomagenesis has been somewhat frustrated by the realization that none of the chromosomal abnormalities could be demonstrated to be specific for MM, nor present in more than approximately 30% of MM patients (Avet-Loiseau et al., 1998; Rao et al., 1998; Sawyer et al., 1998; Rajkumar et al., 1999). Two principal reasons may be at the heart of this problem. Instead of being a single disease entity, MM may constitute a group of diseases that utilize a variety of initiating and tumor progression mutations (corresponding to the observed diversity of cytogenetic changes) before converging on the relatively homogeneous clinical phenotype of MM. The *Intergroupe Francophone du Myélome* has recently supported this view by proposing to distinguish between the pathogenetic pathways that lead to “de novo MM” and “secondary MM,” the latter presumably derived from monoclonal gammopathy of undetermined significance (MGUS) by the loss of a tumor suppressor gene on chromosome 13 (Avet-Loiseau et al., 1999a,b). Alternatively, MM may be a single disease, but the primary genetic event, that could be either linked or unlinked to a cytogenetically detectable alteration, has thus far remained obscure.

This postulate implies that the currently known chromosomal aberrations encompass no more than an assortment of tumor progression events that can be used interchangeably to produce a similar disease phenotype.

Although mouse models of malignant plasma cell tumor development do not completely mimic MM, they may be helpful for developing paradigms with which the above-outlined possibilities can be contemplated. Inflammation-induced plasmacytomas (PCTs) that evolve in the peritoneal cavity of BALB/c mice (Anderson et al., 1969; Potter et al., 1994) offer a model of MM that is widely used, yet imperfect in terms of human disease representation due to major differences in the natural history of PCTs and MM; e.g., PCTs show no bone marrow involvement, have a preference for IgA production, and are dependent in their development on an artificially generated granulomatous tissue. Nevertheless, PCTs may be particularly useful for cytogenetic comparison to MM, because plasmacytomagenesis is genetically defined by virtue of taking place in an inbred mouse strain. This eliminates concerns about genetically determined disease heterogeneities that are likely to be present in outbred populations like human ones. Furthermore, BALB/c PCTs are, unlike MM, well characterized

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with respect to the mutations that initiate oncogenesis. Virtually all tumors (Potter et al., 1992) and tumor precursors (Janz et al., 1993; Kovalchuk et al., 1997) contain a *Myc*-activating translocation, either the common T(12F1;15D2) or the variant T(6C1;15D2). In contrast, rearrangements of *Myc* seem to be secondary events in MM, taking place late during tumor progression (Shou et al., 2000). Based on these considerations, we wondered whether the highly defined and uniformly initiated pathway of malignant plasma cell transformation in the BALB/c mouse was accompanied by equally defined (i.e., consistent or highly recurrent) chromosomal abnormalities that occurred as potential tumor progression events subsequent to the *Myc*-activating translocations, or whether mouse plasmacytomagenesis was, analogous to MM, characterized by a diversity of moderately recurrent and sporadic secondary aberrations.

We utilized mouse SKY (spectral karyotyping) (Liyanage et al., 1996) to assess the chromosome complement of 29 primary PCTs for the presence of primary and secondary cytogenetic alterations. The following key findings were obtained. First, both products of reciprocal translocations known to result in the activation of *Myc* were detected in all tumors: 20 (69%) PCTs harbored a T(12F1;15D2) and 9 (31%) PCTs contained a T(6C1;15D2) (Table 1, column 3). The observation that all tetraploid tumors carried two or more copies of the translocated chromosomes (Table 1, columns 2 and 4) demonstrated that these translocations preceded tetraploidization, a highly recurrent tumor progression event during plasmacytomagenesis (Wiener, 1984). Second, 27 of 29 (93%) PCTs contained additional aberrations that involved all chromosomes except the Y. The majority of the observed changes occurred sporadically, most likely reflecting generalized genomic instability (Table 1, column 8). In contrast, additional copies of chromosome (Chr) 1 and internal duplications of bands 1B/C were detected, respectively, in 28% (8/29) and 24% (7/29) of PCTs (Table 1, column 7; Fig. 1F). This observation suggested that bands 1B/C-derived tumor progressor gene(s) facilitated plasmacytomagenesis in 48% (14/29) of all tumors. Third, aberrations of Chr 5 were uncovered in 15 of 29 (52%) tumors: 13 PCTs harbored non-reciprocal translocations joining Chr 5 with a variable partner chromosome (Chr 2, 4–8, 11, 12, 14, 16, X); one tumor, TEPC 3610, bore a balanced T(5F;5B) translocation; and two other tumors, SIPC 3308 and TEPC 12, contained internal duplications of Chr 5 (Table 1, column 5; Fig. 1A and B). One tumor,

SIPC 3296, harbored two subclones with distinct Chr 5 translocations. Of significance, all but one PCT harbored a single copy of the altered Chr 5 (TEPC 725 contained two), suggesting that rearrangements of Chr 5 occurred subsequent to tetraploidization (Table 1, column 6). The comparison of SKY-painted chromosomes with their inverted DAPI images suggested that the translocation breakpoints on Chr 5 mapped to band F. This was confirmed by FISH analysis of three tumors with large-insert probes hybridizing to *Clock* (at 43 cM on the combined linkage map of Chr 5), *Gnrhr* (44 cM), and *Fgf5* (55 cM) (Fig. 1D), and it was further supported by G-banding of a balanced T(5F;9A) that occurred in one of our archival (not SKY-painted) primary PCTs, SIPC 3388 (Fig. 1E). These findings, taken in conjunction with the presence of similar band 5F rearrangements in long-term established PCT lines (Fig. 1C) (Coleman et al., 1997), led us to believe that band 5F harbors a PCT progressor gene that may become activated during tumorigenesis by non-reciprocal promiscuous translocations. Alternatively, the deletion of the telomeric region of Chr 5 distal to band 5F, that was common to all but three tumors (SIPC 3308, TEPC 12, TEPC 3610), may signal the loss of a putative PCT suppressor gene that resides on the telomeric portion of Chr 5. This situation would be reminiscent of the loss of the putative suppressor gene on Chr 13 in “secondary MM”, as mentioned in the introduction paragraph.

The present study has shown that plasmacytomagenesis in BALB/c mice is a model of malignant plasma cell tumor development that permits one to distinguish clearly between primary cytogenetic changes (*Myc*-activating translocations) and secondary ones (alterations of Chr 1 and 5). Although genetically defined (inbred mouse strain) and uniformly initiated (deregulation of *Myc*), PCTs seem to be remarkably similar to MM with regard to cytogenetic heterogeneity. That is, in many cases sporadic aberrations that presumably reflect genomic instability seem to coexist with recurrent aberrations that may indicate putative tumor progression mutations. Two PCTs, however, were exceptional in lacking any cytogenetic rearrangements besides the T(12F1;15D2) translocation, TEPC 543 and TEPC Sul 2-6. These tumors, which resemble cases of MM that are also characterized by the presence of just one cytogenetic alteration; e.g., a t(11;14)(q13;q32), were probably harvested fortuitously before karyotypic evolution, one of them even before undergoing tetraploidization. Their very existence is intriguing, because it

TABLE I. Summary of Chromosomal Aberrations Detected by SKY in Primary BALB/c Plasmacytomas

PCT ^a	Ploidy ^b	Myc ^c	Copies ^d	Chr 5 ^e	Copies ^f	Chr 1 ^g	Additional aberrations ^h
SIPC 3396	4n	T(12F1;15D2)	2	T(5F;12D)	1	DupB/C	—
SIPC 3424	4n	T(12F1;15D2)	3(4 ^{h-i})	T(5F;2D/E) ^{h-i}	1	+1	Rb(15A1,15A1)[T(15D2;12F1), T(15D2;12F1)] ⁻⁸
SIPC 3428	4n	T(12F1;15D2)	2	T(5F;7E3/F1)	1	+1	Rb(4A1,4A1), +4
SIPC 3449	4n	T(12F1;15D2)	2	T(5F;16B) ^{h-i}	1	—	Rb(11A1,11A1), Rb(17A1,17A1)
SIPC 3489	4n	T(12F1;15D2)	2	T(5E/F;4A1) ^{h-i}	1	+1	T(X[E/F];6D), Del 14 (B-D)
TEPC 54	4n	T(12F1;15D2)	2	T(5F;11B4/5)	1	—	Rb(11A1,11A1), +11
TEPC 420	2n+4n	T(12F1;15D2)	1(2 ^{h-i})	T(5F;X[F]) ^{h-i}	1	DupB/C	Rb(16A1,16A1), -16
TEPC 725	4n	T(12F1;15D2)	2	T(5F;X[A5/6])	2	—	—
TEPC 836	4n	T(12F1;15D2)	2	T(5F;6D)	1	—	Del 6 (B-F), T(14B;8C) ^{h-i}
TEPC 1245	2n+4n	T(12F1;15D2)	2(3 ^{h-i})	T(5F;11B4/5)	1	+1 ^{h-i}	-8, T(11B4/D;2H) ^{h-i} , Rb(6A1,6A1) ^{h-i}
TEPC 3610	4n	T(12F1;15D2)	2	T(5F;5B) ^k	1	—	Rb(17A1,17A1) ^{h-i} , T(9E4;7D) ^{h-i} , T(11A;6B) ^{h-ii} , T(3A4/B;10F) ^{h-iii} , T(4;Del 4[A2-E])(A;A1) ^{h-iv}
SIPC 3228	4n	T(12F1;15D2)	2	—	—	DupB/C, +1	Del 12 (A2-F) ^{h-i}
SIPC 3304	2n+4n	T(12F1;15D2)	1-2	—	—	DupB/C	Rb(11A1,11A1), -8, T(14C;7D) ^{h-i}
SIPC 3340	4n	T(12F1;15D2)	3(2 ^{h-i})	—	—	+1, DupE/F	Rb(X[A1],X[A1]), -8
SIPC 3441	4n	T(12F1;15D2)	2	—	—	DupB/C +1 (+1+1 ^{h-i})	T(7F;12F), T(12F;7F), Dic 18 (C,C) +11
SIPC Sil 1-4	4n	T(12F1;15D2)	2	—	—	—	T(9E4/F;4C7/D1) ^{h-i} , -8
TEPC 543	4n	T(12F1;15D2)	2	—	—	—	—
TEPC 937	2n	T(12F1;15D2)	1	—	—	—	Del 4 (C-D)
TEPC AP	4n	T(12F1;15D2)	2	—	—	—	T(13D;6B), Rb(9A1,9A1), +9, +9
TEPC Sul 2-6	2n	T(12F1;15D2)	1	—	—	—	—
SIPC 3291	4n	T(15D2;6C1)	2	T(5F;14D) ^{h-i}	1	T(1H;1B/C)	Dic 14 (B,B), Del 4 (C-D), T(12C;8D2)
SIPC 3296 ^j	4n	T(15D2;6C1)	3	T(5F;8B/C) T(5F;X[A/B])	1 1	— —	Dup 11 (D), T(19B;7C) ^{h-i}
SIPC 3308	4n	T(15D2;6C1)	3	T(Dup5E/F;7F)	1	—	Dic 14 (C,C), -8, T(12B;X[A]) ^{h-i} , T(4;Del 18[B-D])(C;A) ^{h-i} , T(2H; 4D) ^{h-i} , T(X[F];7E/F) ^{h-i} , T(1H; 16B) ^{h-ii} , T(X[F];3F3) ^{h-iii} , T(18C;3D/E) ^{h-iii}
TEPC 12	4n	T(15D2;6C1)	2	Dup 5(E/F)	1	—	Rb(6A1,6A1)(Del 6[B-D]); 6)T(6C1;15D2)
TEPC 2372	2n	T(15D2;6C1)	2	—	—	+1	Del 8(A4-B2), +9, +16
TEPC 2486	4n	T(15D2;6C1)	2	—	—	DupB/C	T(X[F];7E/F), -X, T(18C/D; 4C) ^{h-i}
SIPC 3282	4n	T(15D2;6C1)	2	—	—	—	+2
SIPC 3287	4n	T(15D2;6C1)	2	—	—	DupB/C	Dic(19C;7A/B), Dic 19(C2/ C2), Del 19(B-C)
SIPC 3338	4n	T(15D2;6C1)	3	—	—	—	T(4C/D;X[F]), Trisomy 6, -8, +2, +3 T(13E;18D) ^{h-i} , Dic (1F/G;7C/D) ^{h-ii}

^aPrimary peritoneal plasmacytomas (PCTs) were induced by intraperitoneal applications of silicone gels (SIPC tumors) or 2,6,10,14-tetramethylpentadecane (TEPC tumors) and analyzed by SKY in consecutive order. No attempt was made to select for or exclude any particular tumor. 10 to 20 matching karyotypes were required for reliable karyotyping. In spite of the technical challenge to collect a sufficient number of metaphase plates from a tumor that is notorious for a low mitotic index, we succeeded in analyzing all but one of the PCTs at the G₀ stage; only TEPC 3610 had to be passaged three times in pristane-primed BALB/c mice (G₁-G₃) before the karyotype could be fully established.

^bPloidy was determined by DAPI-banding: 2n, diploid; 4n, tetraploid; 2n+4n, clonal mix of diploid and tetraploid cells.

^cType of Myc-activating chromosomal translocation. The more common T(12F1;15D2)⁺ tumors are listed prior to the variant T(6C1;15D2)⁺ tumors.

^dCopy number of the chromosome that contained the constitutively active Myc gene, chromosome T(12F1;15D2) or chromosome T(15D2;6C1). Three tumors contained subclones with one additional copy and one tumor harbored a subclone with one copy less (indicated in parentheses).

^eType of Chr 5 rearrangement. Tumors with Chr 5 rearrangements are listed before tumors without such rearrangements.

^fCopy number of the aberrant Chr 5.

^gRearrangements of Chr 1. Internal duplications of bands 1B/C are indicated as DupB/C. The presence of an additional copy of Chr 1 is indicated by +1. SIPC 3441 contained a subclone with two extra copies (indicated by "+1+1"), which rendered the clone trisomic. SIPC 3291 and SIPC 3340 were unusual as they harbored a translocation joining two Chrs 1 and a duplication of bands 1E/F, respectively.

^hAdditional alterations were observed as one-copy events in all cases except the following ones, which were present in duplicates: T(4A;Del4[A2-E])(A;A1) in TEPC 3610, T(13D;6B) in TEPC AP, T(X[F];7E/F) and T(18C/D;4C) in TEPC 2486, T(4C;X[F]) in SIPC 3338.

ⁱPresence of subclones with unique aberrations not observed in the parental clone. The superscripts^{h-i,ii,iii,iv} indicate subclones of decreasing prevalence; i.e., ^{h-i} designates a change found in the most prevalent subclone, ^{h-ii} an aberration present in the second most prevalent subclone, etc.

^jTwo separate clones that harbored distinct aberrations of Chr 5 were observed.

^kBoth products of the reciprocal genetic exchange between Chrs 5 were found.

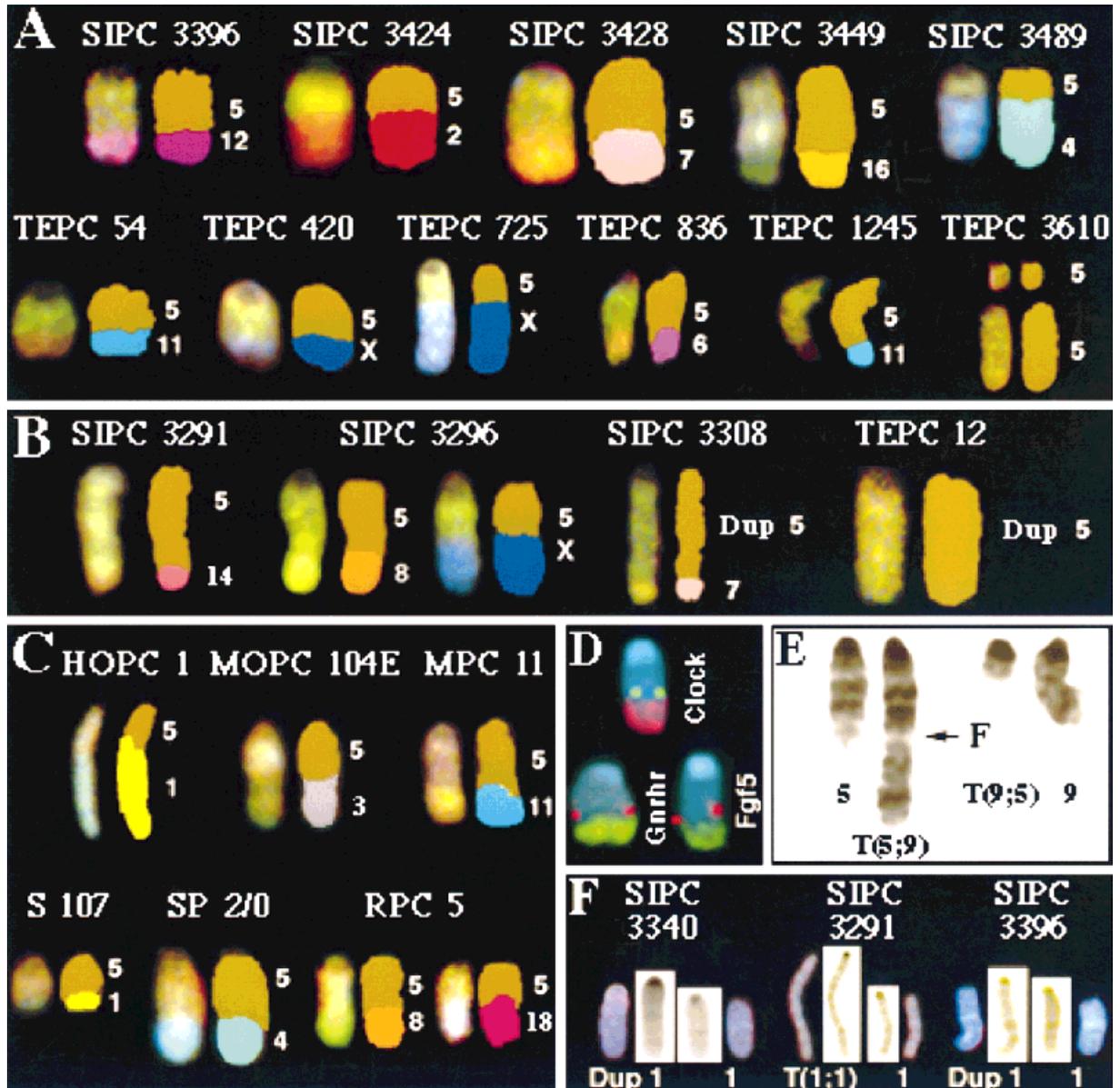


Figure 1. Recurrent alterations of Chr 1 and 5 in primary PCTs. Rearrangements of Chr 5 in primary $T(12F1;15D2)^+$ PCTs, primary $T(6C1;15D2)^+$ PCTs, and established $T(12F1;15D2)^+$ PCT lines are shown in panels **A**, **B**, and **C**, respectively. The majority of Chr 5 aberrations were non-reciprocal translocations with various partner chromosomes. Translocations with Chr 1, 4, 7, 8, and 11 occurred twice and exchanges with the X chromosome 3 times. A single translocation, $T(5F;5B)$ in TEPC 3610, took place as a reciprocal recombination. One primary tumor, SIPC 3296, and one tumor line, RPC 5, contained two cell clones that harbored distinct Chr 5 translocations. Internal duplications of Chr 5 were found in SIPC 3308 and TEPC 12. Translocation breakpoints were mapped to band 5F with the help of FISH probes for *Clock* (circadian locomotor output cycles kaput), *Gnhr* (gonadotropin releasing hormone receptor), and *Fgf5* (fibroblast growth factor 5) after hybridization to the Chr 5-derived portion of

chromosome $T(5F;11B)$ in TEPC 54 (panel **D**). The Chr 5-derived portion and the Chr 11-derived portion of this hybrid chromosome were visualized, respectively, with DAPI in blue and a Chr 11-specific painting probe in red (after labeling with SpectrumOrange) or green (after labeling with rhodamine 110). FISH probes were labeled with SpectrumOrange (*Gnhr*, *Fgf5*) or rhodamine 110 (*Clock*). The location of the breaksite in band 5F was clearly visible in both products of the reciprocal $T(5F;9A)$ found in SIPC 3388, an archival (not SKY-painted) primary plasmacytoma (panel **E**). Shown in panel **F** are SKY and inverted DAPI images of Chr 1 rearrangements; i.e., a representative example of the duplications of bands B/C, as seen in SIPC 3396, the duplication of bands E/F observed once in SIPC 3340, and the $T(1H;1B/C)$ present in SIPC 3291. To facilitate comparison, aberrant Chr 1 are displayed next to SKY and inverted DAPI images of normal Chr 1 that were co-present in the same metaphase spreads.

suggests that plasmacytomagenesis may be completed in the absence of secondary chromosomal rearrangements detectable by SKY. The recent

progress in the molecular cytogenetics of MGUS, smoldering MM, and early-stage MM (Nishida et al., 1997; Avet-Loiseau et al., 1998, 1999a,b) may

soon demonstrate whether this "virgin" mouse plasmacytoma cell has an equivalent in human disease. Similarly, the identification of the mouse PCT progressor genes on Chr 1 and 5, that is currently being pursued in our laboratory, may enable us to evaluate in the not so distant future whether mouse plasma cell malignancies share certain tumor progressor genes with human plasma cell tumors.

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