Jumping Translocation Breakpoint Regions Lead to Amplification of Rearranged *Myc*

To the Editor:

The amplification of cellular proto-oncogenes, a common feature of malignant tumors, results in distinct cytogenetic alterations in carcinomas and hematopoietic neoplasms. In carcinomas, it mainly produces elongation of chromosomes by homogeneously staining regions or extrachromosomal elements referred to as double minutes. In hematopoietic malignancies, in which homogeneously staining regions and double minutes are rare, it gives rise to jumping translocations of chromosomal segments containing oncogenes, ie, segmental jumping translocations (SJTs). SJTs were detected recently in treatment-related leukemias in humans.[1] They have been shown to relocate chromosomal regions harboring the ABL, MLL, INT-2, and MYC oncogenes to one or more recipient chromosomes, creating, thereby, structurally abnormal chromosomes, unidentifiable marker chromosomes, and partial polysomy of the amplified chromosomal segment. Thus far, SJTs have been demonstrated to lead only to the amplification of unrearranged oncogenes in normal genomic configuration. We describe here a new type of SJT that appears to lead to the multiplication of rearranged and activated (ie, constitutively transcribed) oncogenes. The new SJT was observed in the BALB/c mouse plasmacytoma, MOPC 315, in which it effected the transposition onto two marker chromosomes of a chromosomal segment that contained the same clonotypic MOPC 315-typical T(12;15) translocation breakpoint region. This region is known to harbor a recombined and transcriptionally deregulated *Myc* gene.

Spectral karyotyping (SKY)[2,3] was used to analyze the chromosome complement of a subline of the inflammation-induced mouse plasmacytoma, MOPC 315. It readily identified the Chr T(12;15), the hallmark chromosome of BALB/c plasmacytomas, which is thought to be essential for these tumors, because it contains the transcriptionally deregulated *Myc*.[4] In addition, SKY showed the presence of two chimeric marker chromosomes that contained small hybrid segments of Chr 12- and Chr 15-derived material, the Chrs T(12;15;16;12;15;16) and T(17;15;16;12;15;16). The marker chromosomes were of particular interest, because they could be considered as tripartite chromosomes that were produced by joining Chrs 12, 15, and 16, and Chrs 17, 15, and 16, respectively, and then inserting a chromosomal segment that harbored a T(12;15) breakpoint region (Fig 1A). To test this hypothesis, the Chr T(12;15) and the two marker chromosomes were separated by flow sorting.[5] The genomic DNA obtained from flow-sorted chromosomes was amplified by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR)[6] to prepare fluorescence in situ hybridization (FISH) probes for the reverse painting of normal mouse chromosomes. Reverse painting was used to determine the purity of the flow-sorted chromosomes and to confirm and map their composite nature as seen by SKY (Fig 1B). To demonstrate that all three flow-sorted tumor chromosomes contained the MOPC 315-typical translocation breakpoint region, a clonotypic junction fragment between *Myc* and the switch region of the Ig heavy-chain α locus was generated. This was accomplished by PCR using DNA obtained from the flow-sorted chromosomes as template. The DNA sequence analysis of the PCR fragments showed the presence on all three chromosomes of the same T(12;15) translocation breakpoint region previously determined to be unique for MOPC 315[7] (Fig 1C). These findings were interpreted to mean that in MOPC 315 the identical *Myc* rearrangement was relocated by SJTs to two marker chromosomes.

The mechanism of the SJT that amplified the T(12;15) breakpoint region in MOPC 315 is not known. Possibilities include, first, the involvement of extrachromosomal precursors, such as episomes or double minutes containing T(12;15) translocation segments, before a postulated chromosomal reintegration event; second, the occurrence of illegitimate, nonreciprocal, trans-chromosomal recombinations between hyperreplicative or fragile sites; and third, the involvement of recombinogenic repetitive sequences at the breaksites. The latter explanation is supported by findings that breakpoints of jumping translocations, the next close relatives of SJT, are usually found at sites of repetitive DNA, eg, in centromeres or pericentromeric heterochromatin,[8] telomeres, subtelomeric regions, variant telomeric repeats or interstitial telomeric sequences,[9-11] or constitutive heterochromatin[12,13]; however, this has not been shown for MOPC 315. Furthermore, it is conceivable that the *Myc* gene facilitates its own amplification via SJTs as a consequence of a *Myc*-induced mutator phenotype. This hypothesis is based on the proposal that *Myc* acts as a mutator gene in plasmacytomas[14] and the finding that chromosomal translocations were induced by another oncogene, the SV40 large T-antigen.[15]

In conclusion, it is suggested that SJTs may be not only a mechanism for increasing the copy number of unrearranged oncogenes, but also a tumor progression mechanism that leads to the amplification of rearranged, transcriptionally active oncogenes. Additional studies are warranted to determine the prevalence of SJTs in mouse plasmacytomas and to explore if recombined oncogenes can jump in human leukemias and lymphomas, too.

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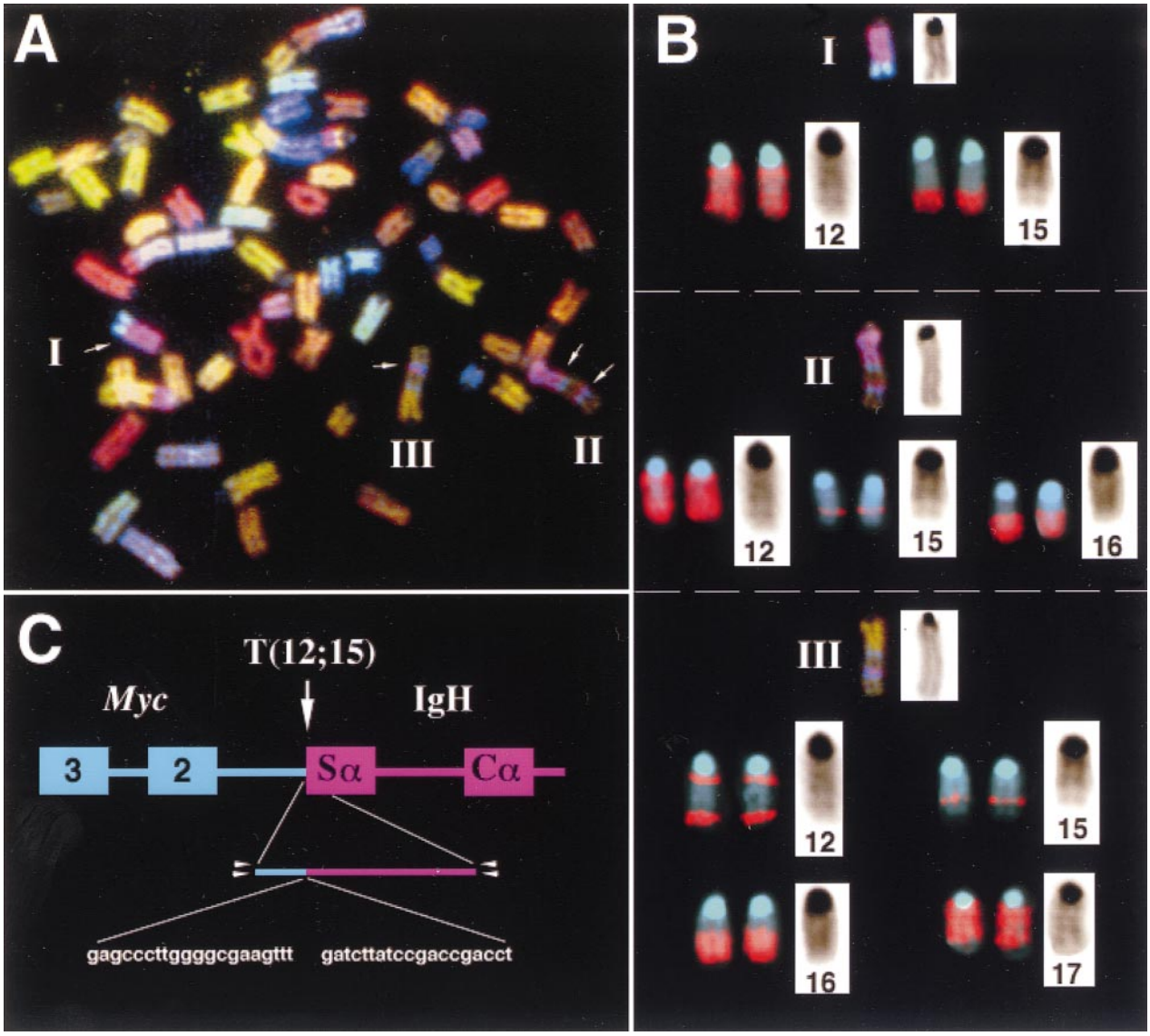

Fig 1. Amplification of T(12;15) translocation breakpoint regions by segmental jumping translocation (SJT) in BALB/c mouse plasmacytoma, MOPC 315. (A) SKY display image of MOPC 315. Rearrangements between Chrs 12 and 15 (indicated by arrows) were observed consistently in 30 images on both the plasmacytoma-specific chromosome T(12;15) and the marker chromosomes, T(12;15;16;12;15;16) and T(17;15;16;12;15;16). The chromosomes are numbered I, II, and III, respectively. (B) Reverse painting of flow-sorted tumor chromosomes on normal mouse chromosomes stained with DAPI. The normal DAPI-stained chromosomes are shown in the white insets to facilitate the interpretation of the reverse painting results. Three distinct flow peaks designated I, II, and III were identified (not shown). They must have contained the translocated chromosomes I, II, and III (shown in [A]) for the following reasons. Peak "I" contained Chr T(12;15) because the translocation juxtaposed the Ig heavy-chain gene cluster, located on Chr 12F2, to the *Myc* locus, residing on Chr 15D2. Therefore, upon reverse painting, Chr 12 should be labeled completely in red (with the exception of the small telomeric cap distal to band F2 that is not discernible in the image due to its small size), whereas Chr 15 should be labeled from band D2 to the telomere. The observed reverse-painting pattern depicted at the top matched this expectation. Peak "II" contained Chr T(12;15;16;12;15;16), because the FISH probe derived from it stained the distal half of Chr 16, but not Chr 17. Peak "III" contained Chr T(17;15;16;12;15;16), because the FISH probe obtained from it stained both Chr 16 and Chr 17. Thus, the reverse painting pattern of the three flow peaks corresponded to the structure of the translocated chromosomes as predicted by SKY. (C) Detection of the same clonotypic junction fragment between the switch region (Sα) of the Ig heavy chain α gene (Cα) and intron 1 of *Myc* by direct, two-round PCR amplification with nested primer pairs (arrowheads). The identical hybrid fragment (indicated by the two-colored horizontal bar) was obtained when DNA samples prepared from the flow-sorted marker chromosomes I, II, and III were used as templates in three different PCR reactions. DNA sequence analysis confirmed the identity of the *Myc*/Sα breaksite and its flanking regions on all three chromosomes. Twenty basepairs of *Myc* and Sα are shown at the bottom to left and right of the breakpoint, respectively. Exons 2 and 3 of *Myc,* exon 1 of Cα, and Sα are depicted as labeled boxes. The T(12;15) translocation breaksite is indicated by an arrow.

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Relationship Between Levels of Leptin and Hemoglobin in Japanese Men

To the Editor:

Leptin, the ob gene product secreted by adipocyte, decreases food intake while it increases energy expenditure and functions as an important signal for the regulation of body weight.[1-3] The leptin receptor is an isoform of the B219 gene product, a member of the hematopoietin receptor family, which is expressed in very primitive hematopoietic cells.[4] Recent studies showed that leptin plus erythropoietin acted synergistically to increase erythroid development in vitro.[5,6] These findings led us to examine the relationship between the serum levels of leptin and hemoglobin.

We surveyed 708 male workers who were not taking any medication. Information regarding smoking habits, alcohol consumption, and physical activity was obtained by questionnaire and/or from medical records. As for the question regarding physical activity, subjects were asked to choose one from the following four answers: no exercise at all, once or twice per month, once or twice per week, and three times or more per week. Blood was drawn in the morning after a 12-hour or longer fast. Serum leptin and insulin were determined by radio immunoassay (Linco Research Inc, St Charles, MO) and enzyme immunoassay (Dinabot, Tokyo, Japan), respectively.

When the subjects were divided into three groups according to their hemoglobin level (<14.5 g/dL [the lowest quintile], 14.5 to 15.8 g/dL, and ≥15.8 g/dL [the highest quintile]), a negative correlation was observed between the levels of leptin and those of hemoglobin, after being adjusted for age, body-mass index, and physical activity (Table 1). The negative correlation became more apparent after further adjustment for the insulin level. In contrast, when similar analysis was performed to examine the relationship between the levels of leptin and white blood cell counts, no correlation was observed between these two variables after being adjusted for related variables.

Table 1. Relationship Between Levels of Leptin and Hemoglobin in Male Workers

	Hemoglobin (g/dL)		
	<14.5	14.5-15.8	≧14.9
n	148	422	138
Age (yr)	47.7 (0.5)	45.9 (0.3)*	44.6 (0.6)†
Body-mass index (kg/m²)	22.30 (0.2)	23.2 (0.1)†	24.1 (0.2)†
Exercise (times/mon)	2.8 (0.3)	2.5 (0.1)	1.8 (0.2)*
Cigarettes (pieces/d)	13.0 (1.3)	12.6 (0.7)	14.9 (1.4)
Alcohol (mL/wk)	202.0 (15.0)	197.0 (8.3)	203.0 (15.6)
Leptin (ng/mL)	3.37 (0.14)	3.61 (0.09)	3.68 (1.58)
Insulin (μU/mL)	3.93 (0.17)	4.56 (2.24)*	5.40 (0.31)†
Adjusted for age, body-mass index, and exercise			
Leptin (ng/mL)	3.71 (0.12)	3.60 (0.07)	3.34 (0.13)‡
Insulin (μU/mL)	4.33 (0.20)	4.54 (0.11)	5.02 (0.20)‡
Adjusted for age, body-mass index, exercise and insulin			
Leptin (ng/mL)	3.75 (0.12)	3.61 (0.07)	3.27 (0.12)*

Values are mean (SE). *P* values are versus the group with hemoglobin levels of <14.5 g/dL. Statistic analysis was performed using the general linear regression model procedures of Statistical Analysis System (SAS Institute, Cary, NC).

*$P < .01$.

†$P < .001$.

‡$P < .05$.

This is the first epidemiologic study showing an association between the levels of leptin and those of hemoglobin. Wilson et al[7] failed to show such a correlation between leptin and red blood cell count without adjusting for leptin-related variables. The gender-dependent difference in serum leptin may be due to the difference of hemoglobin levels, adding to fat mass and sex hormones.[8]

Erythropoiesis is thought to be regulated by erythropoietin, which, in adults, is produced mainly in kidneys, in response to hypoxia.[9] Leptin production occurs mainly in adipocytes, but there has been no report showing that adipocytes have a sensor for hypoxia. It is interesting that bone marrow contains many adipocytes, the role of which is not clear.

Although the effect of leptin on hematopoiesis may be modest, the results of our epidemiologic study, together with those of previous studies performed in vitro,[5,6] suggest that leptin may play some role in hematopoiesis in humans. Further cross-sectional and prospective