Specific Marker Chromosomes Involving a Translocation (12; 15) in a Mouse Myeloma

By Michihiro C. Yoshida* and Kazuo Moriwaki**

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Recent chromosome banding analyses have evoked a particular interest in relation to specific chromosomal changes for human and animal tumors. Shepard et al. (1974a, b) described certain marker chromosomes which were consistently present in several hypotetraploid mouse myelomas. They were identified as a chromosome #12 with an extra band and a partially deleted chromosome #15 or #18, possibly derived from a single translocation. We studied banded karyotypes of a subline and its cultured line of a near-diploid mouse myeloma MSPC-1 (Moriwaki et al. 1971), and found marker chromosomes similar to those reported by Shepard et al. (1974a).

Materials and methods. A solid form subline P-41 derived from MSPC-1 was transplanted subcutaneously at weekly intervals in BALB/c mice as described previously (Moriwaki et al. 1971). A cultured line P established from P-41 was grown in suspension culture in Leibovitz's L-15 medium containing 10% fetal calf serum and 1 mM glutamine. Subcultures were made at 4-day intervals with an equal part of fresh medium. Solid tumors were minced in a 0.075 M KCl solution containing 0.5 µg/ml colchicine, briefly dissociated by pipetting, and filtered through a layer of cheese-cloth. The cell suspension thus obtained was incubated for 20 min at 37°C and then fixed with acetic acid: methanol (1:3). After repeated centrifugations with a fresh fixative the cells were air-dried on slides. Cultured cells were harvested without colchicine treatment and processed according to the routine air-dry method. The slides were stained with quinacrine mustard (QM) after Caspersson et al. (1971), and after with the combined QM-Hoechst method (Yoshida et al. 1975). Karyotypes were arranged following the proposed standard systems (Committee on Standardized Genetic Nomenclature for Mice 1972, Nesbitt and Francke 1973).

Results. The modal chromosome number was obtained as 41 in both the P-41 and P lines. The stemline karyotype was also indistinguishable between the two lines; they showed all members of

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* Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo.
** Department of Cytogenetics, Institute of Genetics, Mishima, Japan.
the normal mouse complement, while the Y was absent, chromosomes #9 and #13 were trisomic, a copy of #12 had an extra bright band at the distal end and a copy of #15 showed a deletion of the terminal segment. The rearranged chromosomes were designated as markers M1 and M2, respectively (Fig. 1). According to the size and staining property, these markers were interpreted as being resulted from a translocation between a terminal portion of #12 and a distal segment of #15 with break points at 12F2 and 15E (Fig. 2). The karyotype was tentatively formulated as 41, X, −Y, +9, +13, t(12; 15). This karyotype was fairly stable and no apparent deviation was found in both lines, so far as our periodic karyotype analyses during 4 months are concerned.

However, a remarkable side line with new marker chromosomes was noticed in the in vitro line after 22 weeks of passages, although the in vivo line did not show any detectable alteration in the chromosome pattern. The in vitro cell population was eventually replaced

![Fig. 1. A representative karyotype from the in vivo line P-41, after a combined staining with QM and 33258 Hoechst.](image-url)
by the new stemline, which thereafter showed further karyotypic evolution through additional serial in vitro passages. Further noticeable was that the in vitro line which was highly tumorigenic in the mouse for the first 4 months showed a much reduced tumorigenicity after the karyotypic evolution mentioned above. Details of this process will be published elsewhere.

Remarks. Our results strongly suggest that the two consistent myeloma markers which were first described by Shepard et al. (1974a, b) as being consistent with mouse myelomas, are ascribed to a translocation between #12 and #15, though their second interpretation for the involvement of #18, instead of #15, is unlikely. The different banding patterns between #15 and #18 were clearly demonstrated by the combined QM—Hoechst staining, especially for the centromeric bands (Fig. 1). Our findings thus were supplementary for the explanation of the origin of the marker chromosomes, and confirmed their common occurrence in mouse myelomas. Similar specific chromosomal changes have been reported to occur in certain other malignant diseases, such as human chronic myelogeneous leukemia (CML) and in some animal tumors induced by chemical agents (Levan 1973, Rowley 1974), among which recently discovered translocation in the Ph¹-chromosome of CML (Rowley 1973) merits special attention, because of the similarity in the genesis. Although

Fig. 2. Four partial karyotypes showing the genesis of a (12;15) translocation in the in vitro line P.
the possibility of a position effect, as suggested for the Ph\(^1\)-translocation (Borgaonkar 1973), may also be applied to the mouse myeloma, this needs to be proven. Since the number of mouse myelomas analyzed by chromosome banding is still small, many cases should be studied to assess the reality of the specific markers.

It has been shown in polyoma-transformed hamster cells that malignant transformation and its reversion are controlled by chromosomal balance caused by gains or losses of certain chromosomes (Hitotsumachi et al. 1971). It remains unknown whether the genetic imbalances due to the extra copies of #9 and #13 and to the loss of the Y in the present myeloma lines are significant for the expression of cellular malignancy. Studies along this line have been in progress, with attention toward karyotypes in \textit{in vitro} lines in which the tumorigenicity was reduced or lost. Our materials have an obvious advantage in that the karyotypes are considerably stable though minor deviations from the normal mouse complement occurred.

\textbf{Summary.} Banded karyotypes were analyzed in P-41 subline of a mouse myeloma MSPC-1 and its \textit{in vitro} line. Both lines showed consistently a 41, X, -Y, +9, +13, t(12; 15) karyotype. This karyotype was fairly stable in both lines for a period of 4 months, although prolonged \textit{in vitro} passages led to changes of the karyotype and tumorigenicity. Two markers resulting from the translocation (12; 15) were considered to be specific for mouse myelomas, in conformity with the findings by Shepard \textit{et al.} (1974a, b).

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