SHORT COMMUNICATION

A PRELIMINARY NOTE ON THE CHROMOSOMES AND TUMORIGENICITY IN HUMAN-MOUSE SOMATIC CELL HYBRIDS¹⁾

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Received April 5, 1972

Somatic human-mouse cell hybrids have provided a favorable system for linkage assignments, because of the rapid and preferential segregation of human chromosomes in such heterokaryons (Weiss and Green 1967; Migeon and Miller 1968). In an attempt to inquire into the possible relationship between tumorigenicity and karyotypic profiles, an *in vitro* cross was made between a human cell-line, B46M, derived from a Burkitt lymphoma, and a mouse cell-line, LM(TK⁻) C1-1D (hereafter referred to as C1-1D). B46M is known as being capable of forming tumors on heterotransplantation into mice (Mino-wada, personal communication), while C1-1D has no tumor-forming capacity, and is resistant to 5-bromodeoxyuridine and deficient in thymidine kinase (Kit *et al.* 1963). Several hybrids were successfully established by the present author from mixtures of both cell-lines by using a selective medium of Littlefield (1964) containing hypoxanthine, aminopterin, and thymidine (HAT). Since B46M cells grew in suspension and C1-1D cells did not proliferate in HAT medium, the former cells could easily be removed by repeated changes of the medium. Usually hybrid colonies were detected after 2 weeks in HAT medium. They were isolated as clones, each of which was established as a mass culture. These clonal lines were maintained by weekly transfers in HAT medium which was changed twice a week.

B46M showed a normal human diploid karyotype (46, XY), while C1-1D was aneu-ploid showing 49 to 55 mouse chromosomes in which 7 to 10 metacentrics were defined. The quinacrine mustard fluorescence technique after Caspersson *et al.* (1971) has efficiently been adopted for the identification of human chromosomes in hybrid karyotypes. The tumor-forming capacity of hybrid clones was examined on heterotransplantation into cortisone-treated newborn mice of CBA/T6T6 and SWJ strains. As many as 12 hybrid clones were isolated to date. In this communication the results from two clones, C1-7 and C1-12, are to be presented, since they were most extensively investigated.

The first heterotransplantation experiments were on the 10th and 12th passage samples for C1-7 and C1-12, respectively. Both clones showed a considerably reduced cumulative take incidence, 6/18 for C1-7 and 5/20 for C1-12, as compared to the incidence obtained with B46M cells (8/10). Chromosome analyses made on the corresponding *in*

1) Contributions from the Chromosome Research Unit, Hokkaido University.

Fig. 1. Quinacrine-stained metaphase plate from C1-12 of a man-mouse hybrid. Arrows indicate human chromosomes identifiable by the specific banding patterns.

vitro passage samples showed that both hybrid clones retained 15–20 human chromosomes. They were identified as A3, C6-8, C10, C12, D13, D14, E16-18, F19-20, and G21-22 (Fig. 1). At the 32nd passage of C1-7 and the 31st passage of C1-12, cultured cells were again inoculated into conditioned newborn mice. The tumor incidence was further reduced (1/15 in C1-7 and 1/13 in C1-12), and both clones formed only a few small tumor nodules which regressed within 2 weeks. The loss of human chromosomes became more apparent on continued *in vitro* passages, and only a few human chromosomes were found in the second samples. Further studies are requested for a possible correlation between the loss of human chromosomes and a marked decline of tumorigenicity or heterotransplantability in the hybrid clones.

The author wishes to express his cordial thanks to Professor Motomichi Sasaki for his suggestions, criticisms and revision of the manuscript, and to Emeritus Professor Sajiro Makino for careful reading through the manuscript. This study was supported by grants from the Ministry of Education (No. 84578 and No. 92035).

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