Construction of Mouse A9 Clones Containing a Single Human Chromosome (X/Autosome Translocation) via Micro-cell Fusion

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Cell hybrids between hypoxanthine guanine phosphoribosyl transferase (HGPRT)-deficient mouse cell lines (A9 or RAG) and each of 12 different human fibroblasts (GM cells) containing various X/autosome translocations were formed, selected and isolated. Several human chromosomes including an X/autosome translocation carrying HGPRT locus were found in these hybrid cells. To construct A9 cell clones that contain a single X/autosome translocation, micro-cell fusion was undertaken to transfer these chromosomes from the hybrids to A9 cells. Karyotype analysis revealed that most of the resulting micro-cell hybrids contain, in a background of mouse chromosomes, only the human X/autosome translocations which were present in the GM cells used for cell hybridization. Sublines of A9 cells were established containing the following autosomal segments: 1q23→1qter; 1q12→1pter; 3p12→3pter; 3q21→3qter; 11q13→11qter; 11p11→11pter; 11q23→11pter; 12q24→12pter; 16q24→16pter; 17q11→17pter.

Key words: X/autosome translocation — Micro-cell fusion — Monochromosomal library

Studies of somatic cell hybrids formed between tumor cells and normal cells have demonstrated the recessive nature of tumor-associated traits including tumorigenicity and/or *in vitro* transformed phenotypes.¹⁻⁴⁾ When tumor cells are fused to normal cells, tumor phenotypes are suppressed. The re-expression of tumor phenotypes correlates with the loss of specific normal cell chromosomes. Thus, putative tumor-suppressor genes have been localized on those normal chromosomes.⁵⁻⁷⁾ A direct approach to identify the chromosome carrying tumor suppressor gene(s) is the introduction of individual normal chromosomes into tumor cells via micro-cell fusion. Saxon *et al.* first demonstrated the complete suppression of tumorigenicity of Hela cells by introduction of a single human chromosome via micro-cell fusion.⁸⁾ A successful transfer of a particular single chromosome to other cells via microcell fusion is substantiated by using chromosome donor cells which have two major characteristics. Firstly, a large number of small-sized micronuclei should be induced in the donor cells by colcemid treatment. Secondly, the chromosome to be transferred should be marked with a selectable gene. Human fibroblasts possessing an X/autosome translocation are useful donors of normal human chromosomes because the X/autosome translocation carrying HGPRT locus could be selectively transferred to HGPRT-deficient cells via micro-cell fusion. However, one shortcoming of the use of such human fibroblast cells as micro-cell donors is the difficulty of inducing a sufficient number of micro-

nuclei for micro-cell fusion. In this study, we made somatic cell hybrids between human fibroblast cells having an X/autosome translocation and HGPRT-deficient mouse cell lines (A9 or RAG). Then, since micronuclei were efficiently induced in these hybrid cells, micro-cell fusion was performed to transfer X/autosome translocations from the hybrids to mouse A9 cells. Several mouse A9 clones which had a single human X/autosome translocation that contains HGPRT locus were constructed. These clones can provide chromosome donors for other chromosome transfer experiments, and thus, should be useful for identifying human chromosomes carrying putative tumor-suppressor genes.

MATERIALS AND METHODS

Cells A9 cells, HGPRT-deficient derivatives of mouse L cells, were kindly provided by the Japanese Cancer Research Resources Bank. RAG cells, HGPRT-deficient mouse cells, were obtained from the American Type Culture Collection. Human fibroblasts, GM 4618, GM0097, GM 0439, GM 1533, GM 0194, GM 1695, GM3322, GM2859, GM3552, GM2621, GM3884 and GM7151 were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). All the cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). Mycoplasma contamination was checked and found to be negative.

Cell hybridization Cell hybrids were formed by the methods described previously.³⁾ Briefly, 10⁶ cells of each

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parental cell line were mixed and cultivated for 2 days. Cell fusion was mediated by a 1 min treatment in 5 ml of 41.5% polyethylene glycol (PEG) (MW 1000, Baker Chemical Co., Phillipsburg, NJ) containing 15% dimethyl sulfoxide followed by the addition of an equal amount of 25% PEG for 1 min. The PEG solution was aspirated and cells were extensively washed with serum-free DMEM. After one day of cultivation in DMEM containing 10% FCS, cells were trypsinized and resuspended in selective medium containing hypoxanthine/aminopterin/thymidine (HAT) plus ouabain ($10^{-5}M$). After 4 to 6 weeks of cultivation, several hybrid clones were isolated, expanded and karyotyped. The hybrid clones which contained the expected X/autosome translocations were chosen as micro-cell donors.

Micro-cell fusion Hybrid cells (3×10^5) were inoculated into 25 cm² flasks (Costar No 3025, Cambridge, MA) and incubated for two days. The micronuclei were induced by colcemid (0.05 μ g/ml) for 48 h in DMEM containing 20% FCS and HAT. After filling flasks with serum-free DMEM containing 10 μ g/ml of cytochalasin B (Sigma Chemical Co., St. Louis, MO), enucleation was performed by centrifugation of the entire flasks placed in acrylic inserts filled with tempered water⁹) in a fixed-angle rotor at 10,000g. All procedures were performed at 34°C. After 1 h of centrifugation, microcells were collected in serum-free medium and the cell suspension was filtered through 8 μ m and 5 μ m polycarbonate filters (Nucleopore, Pleasanton, CA) in series. The purified micro-cells were pelleted by centrifugation at 400g for 10 min and resuspended in serum-free medium containing phytohemagglutinin (PHA) (100 μ g/ml). The micro-cells were attached to prewashed A9 cell monolayers by incubation for 15 min at 37°C. The cells were fused by treatment with 3 ml of 47% PEG (MW 1540) solution for 1 min followed by extensive washing in serum-free medium. After 1 day of incubation in growth medium, cells were trypsinized and plated onto 10 plates (100 mm) containing HAT. Micro-cell hybrids were isolated following cultivation for 10 to 14 days.

Chromosome analysis Conventional chromosome preparations were made by the methods described previously⁴) with minor modifications. The cells were disassociated with phosphate-buffered saline (PBS) containing 0.01% trypsin and 0.02% EDTA. The cells were then pelleted, resuspended in 0.075 M KCl (20°C, 15 min) and fixed in methanol:glacial acetic acid (3:1). The cells were washed twice with fresh fixative before being placed on microscopic slides, air-dried and stained. At least 20 chromosomal spreads/clone were analyzed for the presence of human X/autosome translocations after being banded with quinacrine and stained with Hoechst 33258.¹⁰⁾

Isozyme analysis The cells (10^7) grown in the flasks were trypsinized, suspended in PBS and washed twice with

PBS. Pelleted cells were resuspended in 90 μ l of cell extract buffer containing 10 mM Tris-HCl (pH 7.5), 1 mM MgCl₂, 20 mM KCl, 0.1 mM dithiothreitol and 10% glycerol. Ten μ l of 5% Triton X-100 was added to the cell suspension and the cells were kept in ice for 20 min with occasional mixing. The cells were centrifuged at 13,500g for 30 min at 4°C and the supernatants were collected. For electrophoresis, 2 μ l of cell extract was applied to cellulose acetate plates (Helena Laboratories, Beaumont, TX) and separated for 20 to 60 min at 4°C. After electrophoresis, plates were overlaid with 1% melted agar containing enzyme substrate and a soluble dye which forms colored precipitates upon reaction with the enzyme product. The enzymes examined were peptidase C (PEPC) (EC 3.4), β -galactosidase (GLB1) (EC 3.2.1.23), lactate dehydrogenase A (LDHA) (EC 1.1.1.27), and lactate dehydrogenase B (LDHB) (EC 1.1.1.27).

RESULTS AND DISCUSSION

Table I shows the X/autosome translocations present in the GM cells used in this study. One of the translocated chromosomes which contains the HGPRT gene (Xq26)¹¹⁾ should be transferred to HGPRT-deficient cells by micro-cell fusion. Initial attempts to induce micro-cells from these human cells failed because of the difficulty of inducing sufficient numbers of micronuclei in these cells by colcemid treatment. Therefore, hybrid cells were instead formed between A9 cells or RAG cells and human fibroblasts to be used as micro-cell donors. Cultures were maintained in selective medium containing HAT plus ouabain for 4 to 6 weeks. Hybrid clones formed colonies at a frequency of about 10^{-4} . Five clones were isolated from each combination and one of them was karyotyped by Q-banding plus Hoechst 33258 staining. All the hybrids were found to contain the expected X/autosome translocations carrying the HGPRT locus in addition to several other human chromosomes. Since these hybrid cells were sensitive to the induction of micronuclei by colcemid, micro-cells were prepared by the methods described above. Resulting micro-cells were fused to A9 cells by the use of PEG and micro-cell hybrids were selected in HAT medium for 10 to 14 days. Micro-cell hybrid colonies were formed at a frequency of about 10^{-5} . Three to 10 colonies were isolated from each combination and expanded for karyotyping. As shown in Table I, most of the micro-cell hybrid clones except two clones from GM0097 $\times$ RAG hybrids, which had an unidentified marker chromosome, contain intact X/autosome translocations and no other human chromosomes have been detected. Figure 1A and B shows representative metaphase plates of A9(GM0439)-1 and A9-(GM3552)-2 micro-cell hybrids which contain t(X;3)

Table I. Transfer of a Human X/Autosome Translocation to Mouse A9 Cell Line via Micro-cell Fusion

Human fibroblasts	Translocations	Micro-cell hybrids	Human chromosomes found in micro-cell hybrids
GM4618	t(X;1)(p22;q23)	A9(GM4618)-2, 10	Xqter→Xp22::1q23→1qter
GM0097	t(X;1)(q26;q12)	A9(GM0097)-1 ^{a)}	Xqter→Xq26::1q12→1pter
GM0439	t(X;3)(q26;p12)	A9(GM0439)-1	Xpter→Xq26::3p12→3pter
GM1533	t(X;3)(q26;p12)	A9(GM1533)-1	Xpter→Xq26::3p12→3pter
GM0194	t(X;3)(q28;q21)	A9(GM0194)-3	Xpter→Xq28::3q21→3qter
GM1695	t(X;11)(p21;q13)	A9(GM1695)-3, 4, 5	Xqter→Xp21::11q13→11qter
GM3322	t(X;11)(q22;q13)	A9(GM3322)-1, 3	Xqter→Xq22::11q13→11pter
GM2859	t(X;11)(q11;p11)	A9(GM2859)-3, 4	Xqter→Xq11::11p11→11qter
GM3552	t(X;11)(q26;q23)	A9(GM3552)-2	Xqter→Xq26::11q23→11pter
GM2621	t(X;12)(q22;q24)	A9(GM2621)-4	Xqter→Xq22::12q24→12pter
GM3884	t(X;16)(q26;q24)	A9(GM3884)-1, 2	Xqter→Xq26::16q24→16pter
GM7151	t(X;17)(q11;q11)	A9(GM7151)-3	Xqter→Xq11::17q11→17pter

a) Micro-cell donor was a hybrid clone between mouse RAG cell and GM0097 cell.

and t(X;11) chromosome, respectively. These results indicated that a single human chromosome can be transferred to rodent cells at high frequency without major rearrangements by the methods described here. The stability of a human X/autosome translocation in the mouse A9 micro-cell hybrids cultured in HAT medium was checked. Karyotypic analysis of A9(GM0439)-1, A9(GM3552)-2, A9(GM2621)-4, A9(GM3884)-1 and A9(GM7151)-3 at passage 10 after isolation showed that >93% of the cell population retained the X/autosome translocations. Thus, the human X/autosome translocation is stably maintained in these A9 micro-cell hybrids.

The presence or absence of human isozymes including PEPC, GLB1, LDHA and LDHB was examined in some of the micro-cell hybrids. The results revealed that A9-(GM4618)-2 and A9(GM0097)-1 contain human PEPC, A9(GM0439)-1 and A9(GM1533)-1 contain human GLB1, A9(GM3552)-2 contains human LDHA and A9-(GM2612)-4 contains human LDHB. Since the genes for PEPC, GLB1, LDHA and LDHB are assigned to human chromosome 1q25 or 1q42, chromosome 3p21-cen, chromosome 11p14.1-p15, and chromosome 12p12.2-p12.1, respectively,¹¹⁾ the results confirmed the presence of a portion of human chromosome 1 in A9(GM4618)-2 and A9(GM0097)-1, chromosome 3 in A9(GM0439)-1 and A9(GM1533)-1, chromosome 11 in A9(GM3552)-2 and chromosome 12 in A9(GM2621)-4.

Fig. 1. Quinacrine plus Hoechst 33258 stained metaphase plates of mouse A9 clones containing a single human X/autosome translocation. An arrow indicates the X/autosome translocation. A) A9(GM0439)-1 containing Xpter→Xq26::3p12→3pter. B) A9(GM3552)-2 containing Xqter→Xq26::11q23→11pter.

A library of these micro-cell hybrids containing X/auto-some translocation can provide chromosome donors for other microcell-mediated chromosome transfer experiments. Thus, by using our monochromosomal A9 clones, it is possible to test the presence of putative tumor-suppressor genes of neuroblastoma on #1p,¹²⁾ renal and lung cell carcinoma on #3p,^{13,14)} breast and bladder tumors on #11,^{15,16)} and colorectal tumor on #17.¹⁷⁾ The use of libraries of micro-cell hybrids containing single human chromosome will facilitate human gene mapping and other genetic studies including gene dosage effects, gene deletions, *trans*-acting effects and complementation effects. Such a library can also be used as the source of DNA for a genomic library of human chromosomes.

This system should allow us to construct a whole set of hybrid clones containing individual human chromosomes.

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