

Chromosome 7 Suppresses Indefinite Division of Nontumorigenic Immortalized Human Fibroblast Cell Lines KMST-6 and SUSM-1

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Using nontumorigenic immortalized human cell lines KMST-6 (KMST) and SUSM-1 (SUSM), we attempted to identify the chromosome that carries a putative senescence-related gene(s). These cell lines are the only ones that have been established independently from normal human diploid fibroblasts following in vitro mutagenesis. We first examined restriction fragment length polymorphisms on each chromosome of these immortalized cell lines and their parental cell lines and found specific chromosomal alterations common to these cell lines (a loss of heterozygosity in KMST and a deletion in SUSM) on the long arm of chromosome 7. In addition to these, we also found that introduction of chromosome 7 into these cell lines by means of microcell fusion resulted in the cessation of cell division, giving rise to cells resembling cells in senescence. Introduction of other chromosomes, such as chromosomes 1 and 11, on which losses of heterozygosity were also detected in one of the cell lines (KMST), to either KMST or SUSM cells or of chromosome 7 to several tumor-derived cell lines had no effect on their division potential. These results strongly suggest that a gene(s) affecting limited-division potential or senescence of normal human fibroblasts is located on chromosome 7, probably at the long arm of the chromosome, representing the first case in which a specific chromosome reverses the immortal phenotype of otherwise normal human cell lines.

Normal human diploid fibroblasts in culture exhibit a limited life span and cease to divide after 50 to 70 population doublings (PDs) (6). The time to the cessation of division potential is inversely correlated with the age of the sample, and this cessation of division potential is termed cellular senescence. Escape from this regulation results in immortalized cells capable of proliferating indefinitely. Hybrid cells obtained by fusion between normal and immortal cells exhibit the limited division potential of normal cells, indicating that the normal limited division potential is dominant over the immortal phenotype (4, 13, 19, 20). Complementation analysis with cell-cell hybrids between different immortal human cells demonstrated that immortal phenotypes are classified into at least four (A, B, C, and D) groups, indicating that immortality is derived from a limited number of recessive genetic alterations (21). It is quite likely that cellular senescence is genetically determined rather than caused by random deleterious events.

Several lines of experimental evidence, most obtained by specific chromosome transfer into immortalized cells, support this notion. Transfer of chromosome 1 suppressed the indefinite division potential of Syrian hamster cells (27), a uterine endometrial carcinoma cell line (32), and neuroblastoma cells (2). Introduction of chromosome 4 suppressed the immortal trait of cells belonging to complementation group B (17). Likewise, chromosome 11 suppressed the growth of embryonal rhabdomyosarcoma cells (12). However, it remains to be determined whether the apparent loss of division potential is a consequence of bona fide cellular senescence

or reflects tumor suppression or terminal differentiation induced by the introduced chromosome, since the immortal human cells used in the above experiments were derived from tumor cells which have lost the ability for terminal differentiation and show malignant phenotypes. In contrast, introduction of a specific chromosome into tumor-derived cell lines suppresses their malignant phenotype with or without affecting their indefinite division potential (18, 23, 26). Tumor suppression was thus proposed to represent one mechanism for cellular senescence (18, 23).

Because of the above complications stemming from the use of tumor-related cell lines, the use of immortalized cell lines derived from normal human cells should be highly desirable, particularly for studies on cellular senescence. However, the difficulties in obtaining immortalized cells from normal diploid fibroblasts or epithelial cells, due in part to the recessive nature of immortalization, have long hampered such experiments. Recently, a few immortal human cell lines have been established from normal human cells in culture following repeated exposure to radiation or chemical mutagens. These include cell lines derived from diploid fibroblasts (14, 15), mammary epithelial cells (25), and keratinocytes (29). In an attempt to identify a senescence or mortality gene(s) involved in normal diploid fibroblasts, we used two of these nontumorigenic immortalized human cell lines, KMST-6 (KMST) and SUSM-1 (SUSM), which were established from fetal human fibroblasts after repeated gamma irradiation and 4-NQO treatment, respectively (14, 15). Both cell lines are nontumorigenic in nude mice, do not form colonies in soft agar (14, 15), and belong to the same complementation group with respect to their genetic background of immortalization (21). As a technical advantage in

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pursuing the identification of a putative senescence gene, their parental normal diploid fibroblasts are also available, enabling us to locate sites of loss of heterozygosity (LOH), which is closely associated with the expression of recessive mutations, on specific chromosomes by restriction fragment length polymorphism (RFLP) analysis.

In this article, we show that overlapping chromosomal rearrangements were observed on the long arm of chromosome 7 in both KMST and SUSM cells and that introduction of chromosome 7 specifically suppresses the indefinite division potential of these cell lines. These results strongly suggest that one of the genes involved in the limited division potential (or senescence) of human cells is located on the long arm of chromosome 7.

MATERIALS AND METHODS

Cell lines and culture conditions. The immortal human cell lines KMST and SUSM were established from fetal human diploid fibroblast cell lines KMS and AD387, respectively, by repeated gamma ray irradiation and 4-NQO treatment and are karyotypically hypertetraploid and nearly triploid, respectively, with doubling times of 20 to 24 h (14, 15). The microcell donors contain a single copy of human chromosome 1, 7, or 11, each of which is tagged with the neomycin resistance gene (*neo*), in an A9 mouse fibroblast background (10). The donor cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Sigma, St. Louis, Mo.) and 800 μ g of geneticin (G418; Sigma) per ml to maintain the *neo*-tagged chromosomes (10). KMST (and its hybrids) and SUSM (and its hybrids) were cultured in ES medium (Nissui Seiyaku Co. Ltd., Tokyo, Japan) with 10% serum and Eagle's minimal essential medium with 10% serum, respectively, as described before (20). Microcell hybrids were selected in medium containing 200 μ g of G418 per ml for KMST microcell hybrids and 300 μ g of G418 per ml for SUSM microcell hybrids 2 days after fusion. The tumor-derived cell lines HT-1080 (fibrosarcoma), HeLa (cervical carcinoma), and TE85 (osteosarcoma) were obtained from the Japanese Cancer Research Resources Bank and maintained in ES medium supplemented with 10% fetal calf serum. Their microcell hybrids were selected in the same medium containing G418 (400 μ g/ml).

RFLP analysis. Genomic DNAs were purified, digested with restriction endonucleases (Takara Shuzo Co. Ltd., Kyoto, Japan), and subjected to Southern blot analysis with approximately 60 highly polymorphic 32 P-labeled DNA probes as described before (1). The chromosome-specific DNA probes relevant to this study were pHHH106 (D1S67, 1q21-q25) (3) for chromosome 1; pRM7.4 (D7S370, 7pter-p14) (22), pF-167.2 (D7S252, 7q31-q32) (22), and pJCZ67 (D7S396, 7q35-qter) (22) for chromosome 7; and pADJ762 (D11S12, 11pter-p15.5) (9) for chromosome 11. The probes, including other chromosome 7-specific probes (11), were provided by the Japanese Cancer Research Resources Gene Bank.

Microcell-mediated chromosome transfer. Micronucleation, microcell fusion, and hybrid isolation were performed as described before (10). Hybrid cells were inoculated into 25-cm² flasks (no. 3025; Costar Cambridge, Mass.) and incubated for 2 days. The micronuclei were induced by colcemid (0.05 μ g/ml) for 48 h in Dulbecco's modified Eagle's medium containing 20% fetal calf serum and G418. After the flasks were filled with serum-free medium containing cytochalasin B (Sigma; 10 μ g/ml), enucleation was performed by centrifuging the flasks placed in acrylic inserts

filled with tempered water in a fixed-angle rotor at 10,000 \times g. All procedures were performed at room temperature. 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, Calif.) in series. The purified microcells were pelleted by centrifugation at 400 \times g for 10 min and resuspended in serum-free medium containing phytohemagglutinin (100 μ g/ml). The microcells were attached to washed A9 cell monolayers by incubation for 15 min at 37°C. The cells were fused by treatment with 3 ml of 47% polyethylene glycol 1540 (Baker Chemical Co., Phillipsburg, N.J.) solution for 1 min and extensive washing in serum-free medium. After 1 day of incubation in growth medium, the cells were trypsinized and plated onto 90-mm dishes containing G418 at the indicated concentration.

Polymerase chain reaction. Genomic DNA and cell lysates were prepared from the microcell donors, the recipient cell lines, and microcell hybrids as described before (21, 31). Dinucleotide repeat sequences were amplified from these samples by polymerase chain reaction with microsatellite (dC-dA)_n primers of specific chromosomes. The presence of the donor-type allele of chromosome 7 was detected with primer set Mdf20 (locus D7S435, 7p21-7pter; 5'-TTAAAAT GTTGAAGGCATCTTC-3' and 5'-TTTGAGTAGGTGGCA TCTCA-3') (30, 31). After polymerase chain reaction with the thermal cycle conditions described before (30, 31), amplified dinucleotide repeats were labeled with 32 P-endolabeled primers, resolved in nondenaturing 8% polyacrylamide gels, and autoradiographed as described before (1).

Cytogenetic analysis. The procedures for fluorescent in situ hybridization with R-banded metaphase chromosomes have been described previously (28). Hybrid cells were cultured for 6 h in the presence of 5-bromodeoxyuridine (25 μ g/ml) prior to treatment with colcemid (0.05 μ g/ml) for 90 min. Metaphase spreads were prepared from these cells, stained with 1 μ g of Hoechst 33258 per ml in Sørensen's phosphate buffer (pH 6.8) for 5 min, and then exposed to black light. The R-banded chromosomes were hybridized with biotinylated pSV2neo plasmids, conjugated with goat anti-biotin antibody, and further conjugated with fluorescein-labeled anti-goat immunoglobulin G antibody to amplify the fluorescence signals. The chromosomes were finally counterstained with propidium iodide (0.5 μ g/ml) and microphotographed.

RESULTS

Detection of LOH. As a first step to identifying a specific chromosome(s) which is involved in the immortal phenotype of KMST and SUSM cells, we examined whether LOHs, which are generally associated with the expression of recessive mutations, are present on specific chromosomes in these cell lines. Identification of specific chromosomes on which LOHs are found should facilitate the identification process by limiting the number of chromosomes to be tested. We therefore compared RFLPs on every chromosome for KMST and SUSM cells and their parental diploid fibroblast lines by Southern blot analysis with a total of approximately 60 highly polymorphic DNA markers.

With these probes, LOHs were detected on chromosomes 1, 7, and 11 in KMST cells. Figure 1A shows the disappearance of a 2.1-kb *Msp*I band at a 1q region, a 6.2-kb *Bgl*I band at a 7q region, a 5.2-kb *Rsa*I band at a 7qter region, and a 0.5-kb *Msp*I band at an 11p region in KMST cells. The LOHs did not seem to be the result of chromosome loss but seemed to be confined to a particular region on each of the chromo-

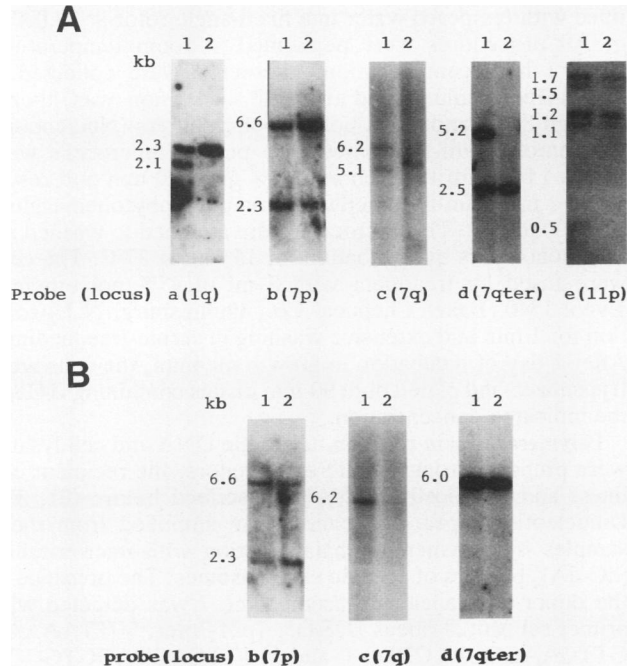


FIG. 1. RFLP analysis of KMST (A) and SUSM (B) cells. (A) Lanes 1, KMS (parent); lanes 2, KMST. (B) Lanes 1, AD387 (parent); lanes 2, SUSM. Below each panel, the probes (a through e) used and their chromosomal loci are indicated. The probes (restriction endonuclease used to detect RFLPs) are: a, PHHH106 (*MspI*); b, pRM7.4 (*MspI*); c, pF-167.2 (*BglI*); d, pJCZ67 (*RsaI*); and e, pADJ762 (*MspI*). The molecular sizes of DNA bands are indicated at the left. For details, see Materials and Methods.

somes, since the DNA sequences detected by other probes were not always homozygous in chromosomes 1, 7, and 11 (data not shown). Recombinogenic events, therefore, must have occurred to generate partial homozygosities (LOHs) in these chromosomes. In SUSM cells, an LOH could not be detected in any chromosome, but a deletion was detected on chromosome 7 with the same probe that revealed an LOH in KMST cells (Fig. 1B, 6.2-kb *BglI* band in the 7q region).

We next conducted more extensive studies on chromosome 7 with probes specific to this chromosome. One probe (D7S252, 7q31-32) revealed an LOH in KMST and a deletion in SUSM cells (Fig. 1, probe c). Another probe (D7S396, 7q35-qter) also revealed an LOH in KMST cells but not in SUSM cells (Fig. 1B). Probe b (D7S370, 7pter-p14) showed heterozygosity in both KMST and SUSM cells. The 7q31-32 region was thus found to be altered in both KMST and SUSM cells. These results suggest that a recessive mutation on the long arm of chromosome 7 is responsible for the expression of the immortal phenotype of KMST and SUSM cells, although the involvement of chromosome 1 and 11 could not be excluded by these experiments alone. The detection of the chromosomal rearrangements on chromosome 7 in both KMST and SUSM cells was quite unexpected, since the presence of tumor suppressor genes or genes relevant to immortalization on this chromosome has not been reported (23, 26). On the other hand, LOHs at the particular regions on chromosome 1 are often observed in various tumor cell lines and have been shown to result in the loss of a tumor suppressor gene (23, 26). The LOH on the short arm of chromosome 11, closely linked to the Wilms' tumor locus, has also been described before (26).

TABLE 1. Division potential of KMST microcell hybrids

Chromosome introduced	No. of microcell hybrids tested	No. or range of PDs achieved before division cessation	No. of clones with arrested division
1	4	>100	0
7	23	10-30 ^a	13 (15) ^b
11	12	>100	0

^a Exact values: <13 PDs, six clones; <18 PDs, two clones; <20 PDs, three clones; <22 PDs, one clone; and <28 PDs, one clone.

^b Two additional clones did not increase in cell number during the initial 4 weeks following fusion but resumed rapid growth thereafter.

Division potential of microcell hybrids. We introduced chromosomes 1, 7, and 11 into KMST and SUSM cells by means of microcell fusion and examined whether their introduction affected the proliferation behavior of these cell lines (Table 1). Microcells containing on average a single metaphase chromosome were prepared from the chromosome donor cells and then fused to KMST and SUSM cells to produce microcell hybrids as described under Materials and Methods. G418-resistant microcell hybrids containing introduced chromosome 1, 7, or 11 arose at frequencies of 1×10^{-6} , 5×10^{-6} , and 5×10^{-6} per treated cell, respectively, in KMST cells (Fig. 2). Microcell hybrids from KMST cells into which chromosome 1 or 11 was introduced did not show any significant change in division potential. Four clones carrying introduced chromosome 1 that were tested continued to grow for more than 100 PDs, although some of them showed a retarded growth rate and a slight change in cell morphology. All 12 microcell hybrids containing introduced chromosome 11, which appeared most frequently, could also be subcultured for more than 100 PDs without any loss of proliferation potential. In contrast, hybrids containing introduced chromosome 7 exhibited limited proliferative potential and ceased to divide, with alteration in the shapes of the cells.

In Fig. 2, photographs of two such clones taken at intervals postfusion are presented. Of the 23 hybrid clones tested, 6 had very limited growth potential, attaining only 300 to 1,000 cells (ca. 8 to 10 PDs) after a 3-week incubation following fusion (Table 1). The cell number of these hybrids did not change even after 2 to 3 weeks of additional culture with weekly changes of medium. During this period, the cells became significantly larger, resembling typical senescent cells. Seven hybrid clones were subcultured for a longer period of time than the six described above but finally ceased to proliferate after achieving 10 to 30 PDs accompanied by enlargement of the cells. Two clones showed limited proliferative division potential for a few weeks following fusion, but variant immortal cells were segregated. These segregated cells probably emerged after rearrangement of chromosomes, which is frequently observed in KMST cells (see Discussion). The growth of the remaining eight clones did not seem to be affected.

We conducted basically the same experiments with SUSM cells. As shown in Table 2 and Fig. 3, the results were quite similar to those obtained with KMST cells. While introduction of chromosome 1 or 11 did not have any significant effect on their division potential, a considerable portion (30 of 44) of the SUSM cell hybrids containing introduced chromosome 7 exhibited a limited division potential. Thus, the two immortalized cell lines, which had been established independently following gamma irradiation or 4-NQO treatment of normal human fibroblasts but were classified in the

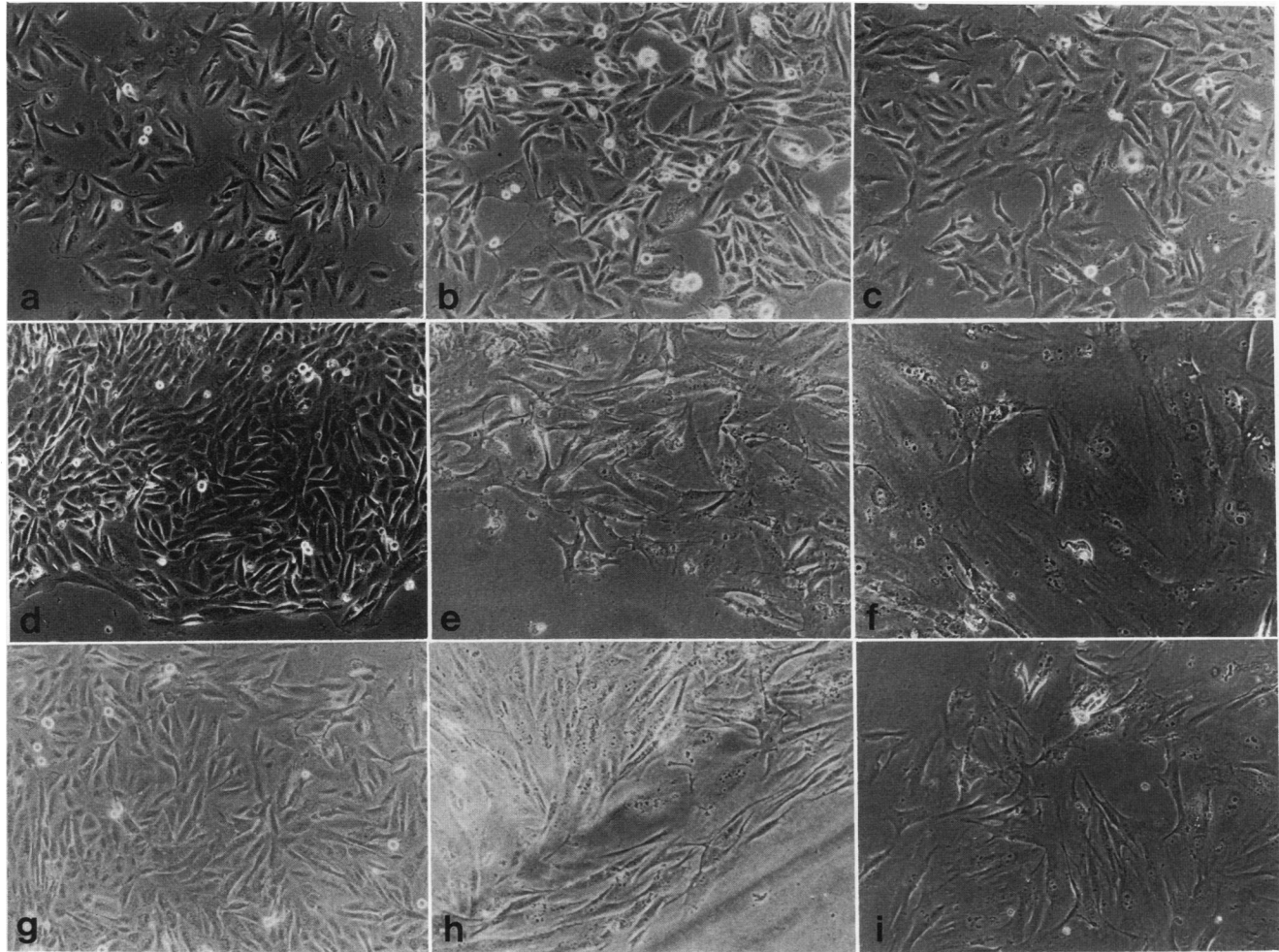


FIG. 2. Phase-contrast micrographs of KMST cells and microcell hybrid clones. (a) KMST; (b) hybrid containing introduced chromosome 1; (c) hybrid containing introduced chromosome 11; (d, e, and f) one hybrid clone containing introduced chromosome 7 cultured for 3, 6, and 8 weeks postfusion, respectively; (g, h, and i) another hybrid clone containing introduced chromosome 7 cultured for 3, 6, and 8 weeks postfusion, respectively.

same complementation group with respect to immortality (15a, 21), responded to the same chromosome (chromosome 7) in reversing their indefinite division potential.

Identification of the introduced chromosomes in microcell hybrids. The presence of the introduced chromosome 7 in the microcell hybrids was confirmed by polymorphism analysis with probes for microsatellite DNA sequences. With chromosome-specific microsatellite primers (30, 31), dinu-

cleotide repeat sequences were amplified from DNA obtained from KMST and SUSM microcell hybrids along with DNA obtained from control cells (microcell donor and recipient cells) and resolved on a polyacrylamide gel. Of the four primer sets tested, one pair of primers, designated Mfd20 (locus D7S435, 7p21-7qter) (30), was found to distinguish the donor allele from the recipient alleles, giving rise to an amplified DNA band of the expected size (approximately 130 bp) of the donor-type allele of chromosome 7 in KMST and SUSM microcell hybrids (Fig. 4). In KMST hybrids, three of four of the suppressed clones and two of five of the unsuppressed clones retained this donor-type allele. In SUSM hybrids, five of five of the suppressed clones and three of five of the unsuppressed clones contained this allele. Figure 4 shows one suppressed hybrid from each recipient. Therefore, at least a portion of the introduced chromosome 7 was retained in the majority of the suppressed hybrids selected by G418 resistance. The presence of the introduced chromosome 1 in the microcell hybrids of KMST and SUSM cells was also confirmed by using a primer set specific to chromosome 1 (not shown). The presence of the donor-type allele of chromosome 11 was confirmed by Southern blot

TABLE 2. Division potential of SUSM microcell hybrids

Chromosome introduced	No. of microcell hybrids tested	No. or range of PDs achieved before division cessation	No. of clones with altered division potential
1	7	>100	0
7	44	10-45 ^a	28 (30) ^b
11	16	>100	0

^a Exact values: ~10 PDs, eight clones; <18 PDs, 13 clones; <20 PDs, one clone; <23 PDs, four clones; <26 PDs, one clone; and <42 PDs, one clone.

^b Two additional clones exhibited a period of distinct slowing in the growth rate before resuming rapid proliferation.

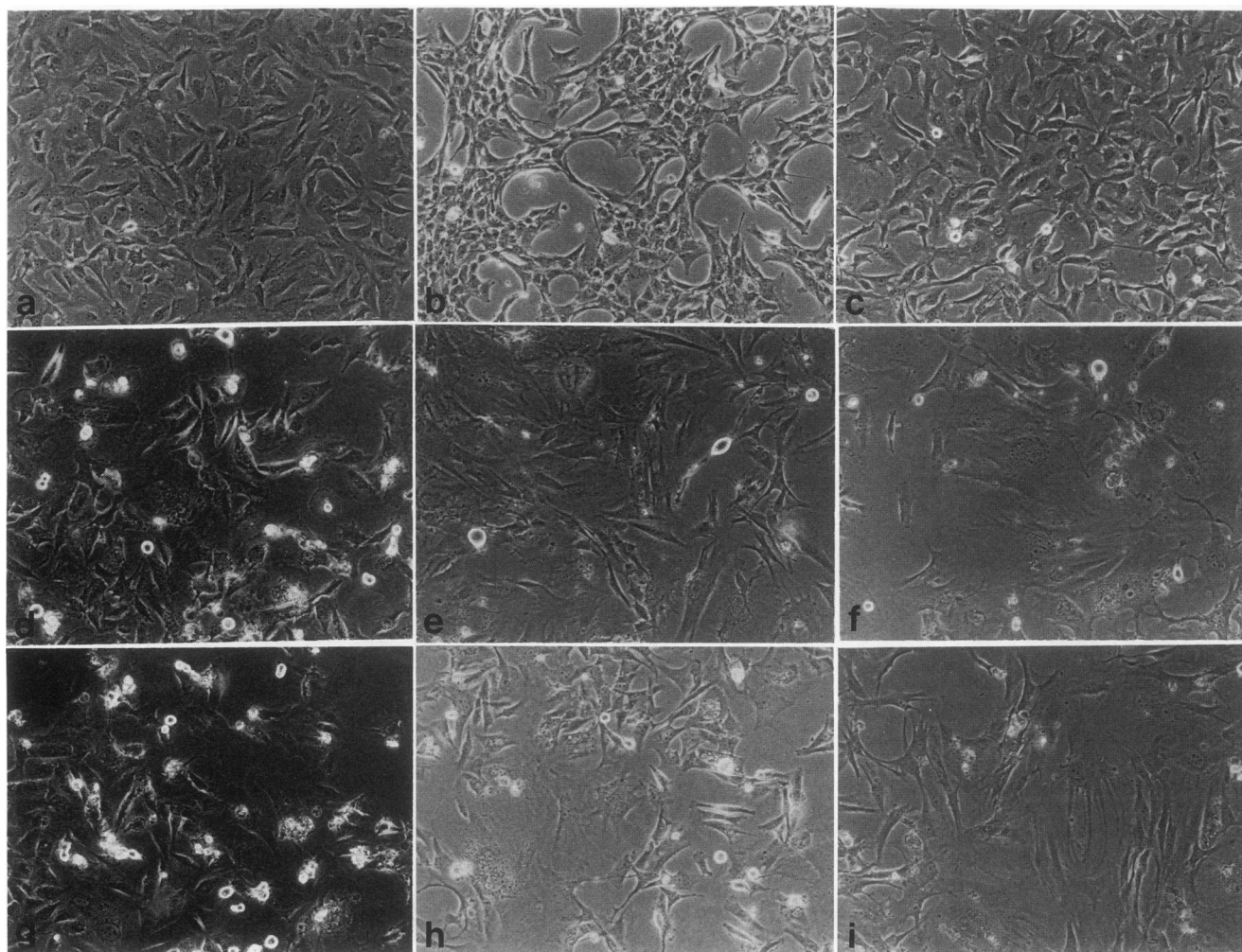


FIG. 3. Phase-contrast micrographs of SUSM cells and microcell hybrid clones. (a) SUSM; (b) hybrid containing introduced chromosome 11; (c) hybrid containing introduced chromosome 7; (d, e, and f) one hybrid clone containing introduced chromosome 7 cultured for 3, 6, and 8 weeks postfusion, respectively; (g, h, and i) another hybrid clone containing introduced chromosome 7 cultured for 3, 6, and 8 weeks postfusion, respectively.

analysis with a polymorphism marker specific to this chromosome (not shown).

The presence of the introduced chromosome 7 in the microcell hybrids was further confirmed by cytogenetic analysis by fluorescence in situ DNA hybridization with the

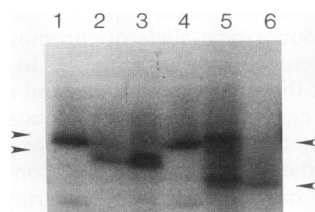


FIG. 4. Detection of introduced chromosome 7 in microcell hybrids by polymerase chain reaction with microsatellite primers. Lane 1, mouse A9 cells containing human chromosome 7 (donor); lane 2, KMST microcell hybrid clone; lane 3, KMST (recipient); lane 4, same as lane 1; lane 5, SUSM microcell hybrid clone; lane 6, SUSM (recipient). The arrowheads indicate the positions of amplified DNA specific to the donor and recipient alleles of chromosome 7.

neo gene as a probe. Aliquots from the cultures of KMST and SUSM hybrid clones which had limited but relatively longer division potential were cultured to prepare R-banded metaphase spreads. Of the eight hybrid clones examined, only five (two KMST hybrids and three SUSM hybrids) displayed typical doublet fluorescence signals (data not shown). In all five of the positive hybrid clones, the signals were localized specifically to the terminal region (q32-33) of the long arm of intact chromosome 7, coinciding with the region determined previously by a radioisotopic method (18a). In every case, no metaphase spread contained more than one copy of the *neo*-tagged chromosome. However, the short arm of the *neo*-tagged chromosome was occasionally deleted to various degrees in individual clones. This observation coincided with the data from the above polymerase chain reaction analysis. The remaining three clones did not give a metaphase spread. Even in the five positive hybrids, the proportion of metaphase cells was extremely low despite prolonged colcemid treatment of the cells.

These results demonstrated clearly that the suppression of division potential in microcell hybrids of KMST and SUSM

cells was due to the presence of a single copy of the introduced chromosome 7.

Effect of introduction of chromosome 7 into other human cell lines. To determine whether the suppression of division potential by the introduction of chromosome 7 was limited to KMST and SUSM cells, we extended the microcell fusion analysis to several tumor-derived immortalized cell lines. The introduction of chromosome 7 into three tumor-derived immortal lines, HT-1080 (fibrosarcoma), HeLa (cervical carcinoma), and TE85 (osteosarcoma), which have been assigned to complementation groups A, B, and C, respectively (21), had no effect on their division potential. Similarly, the introduction of chromosome 11 into these cell lines had no suppressive effect on their indefinite division potential. Although a limited number of cell lines were examined, these results suggest that the effect of chromosome 7 on reversing the indefinite division potential of cells is limited to KMST and SUSM cells among the cell lines so far examined.

DISCUSSION

We have demonstrated that the introduction of normal chromosome 7 reverses the immortal phenotype of the human fibroblast cell lines KMST and SUSM, which are the only nontumorigenic cell lines established in vitro from normal human diploid fibroblasts. The availability of their parental cell lines has made it possible to locate a chromosomal rearrangement on specific chromosomes by RFLP analysis, narrowing down the number of candidate chromosomes to be tested in microcell fusion experiments. In fact, we were able to find two types of chromosomal rearrangements in the same region of chromosome 7 in KMST and SUSM cells, an LOH (7q31-qter) in KMST and a deletion (7q31-32) in SUSM. Furthermore, introduction of chromosome 7 into these cell lines by means of microcell fusion caused the cessation of cell division, giving rise to senescence-like behavior. The introduction of chromosome 7 had no effect on the division potential of several tumor-derived immortal cell lines. This is the first demonstration identifying a chromosome (chromosome 7) which reverses the immortal phenotype of physicochemically mutagenized nontumorigenic human cell lines. The results also suggest that a gene(s) located on the distal part of 7q is responsible for the suppression of the immortal phenotype of these nontumorigenic cell lines.

In our microcell fusion experiments, hybrids whose proliferation potential was affected by the introduction of chromosome 7 constituted approximately 60% of the G418-resistant clones of both KMST and SUSM cells under various selective conditions. Since the chromosomes of these lines are highly rearranged and 70 to 80% of the donor mouse A9 cells usually contain intact *neo*-tagged human chromosome 7, the above proportion (ca. 60%) of hybrids affected in proliferation potential among all G418-resistant clones should not be unexpected. In this respect, the position of the putative immortalizing gene relative to the integration site of the *neo* marker, which is on the tip of the long arm of chromosome 7, must also have affected the yield of clones with limited proliferation potential.

Both KMST and SUSM cells have been assigned to complementation group D from cell-cell hybrid analysis of the immortal phenotype (15a, 21). As described above, the introduction of chromosome 7 into the three tumor-derived immortal cell lines representing the other complementation groups did not affect their proliferation potential. Thus, we are tempted to speculate that a gene(s) responsible for the

immortalization of group D cell lines is located on chromosome 7, which may be a straightforward interpretation of the results presented here. On the other hand, we believe that such a conclusion must be drawn with some reservations. First, for practical reasons, we tested only three chromosomes (1, 7, and 11) in our chromosome transfer experiments. The genetic lesions responsible for the immortal phenotype in KMST and SUSM cells may be located on other chromosomes despite the presence of the overlapping rearrangement on their chromosome 7 and the finding that these cells have responded to chromosome 7. If this is the case, it may follow that chromosome 7 has indirectly suppressed the immortal phenotype of in vitro-mutagenized normal cell lines (not limited to group D cell lines). Since, to date, the number of cell lines established from mutagenized normal human cells is quite limited, it would be very difficult to prove that this is the case. In any event, it should be emphasized that the introduction of the same chromosome (chromosome 7) reversed the immortal phenotype of nontumorigenic KMST and SUSM cells which were independently isolated from normal human fibroblasts through physical and chemical treatment, respectively.

It is interesting that an LOH was also detected on chromosomes 1 and 11 in KMST but not in SUSM cells. Aberrations on these chromosomes are observed in numerous tumor cell lines of various types (26). These observations have led to the idea that aberrations on chromosomes 1 and 11 are involved in tumor development by conferring a growth advantage. At present, these chromosomes have been proved to contain several tumor suppressor genes. Indeed, transfer of these chromosomes is known to suppress the malignant phenotype of certain tumor cell lines with or without affecting their indefinite division potential (see Introduction). However, they had no effect on the proliferation potential of KMST, SUSM, or most of the tumor-derived control cell lines tested. Since KMST but not SUSM cells can be converted to tumorigenic cells by transfection of the activated *ras* oncogene (15), the presence of the LOHs on chromosome 1 or 11 in KMST cells could account for this difference. Recessive genetic alterations of these chromosomes are therefore likely to be involved in the development of malignancy and to have no direct role in immortality.

At present, there seem to be several mechanisms by which the introduction of a normal chromosome into immortal cells suppresses their proliferation and/or division potential. Tumor suppression and induction of terminal differentiation have been implicated in the suppression of proliferation of several tumor-related cell lines by the introduction of specific chromosomes (2, 12, 17, 18, 23, 26, 32). Although the relationship between tumor suppression and terminal differentiation is not well understood, terminal differentiation, in addition to tumor suppression, can be accepted as one mechanism for the cessation of cell division. For example, human keratinocytes can be induced to differentiate, but protracted gamma irradiation generates differentiation-resistant immortalized clones which do not form colonies in soft agar or tumors in nude mice, as observed for KMST and SUSM cells (15). Likewise, an immortal cell line established from mammary epithelial cells by a chemical mutagen has a phenotype quite similar to that of immortalized keratinocytes (25). Additional genetic alterations are apparently required for these immortal cells to acquire a malignant phenotype (7). These results can be interpreted as meaning that a block to terminal differentiation may lead to indefinite division in epithelial cells.

Similar to the above phenomena, differentiated cells such

as keratinocytes and other epithelial cells can be converted to immortalized cells at a substantial frequency following transfection with DNA viruses such as simian virus 40, adenoviruses, and human papillomaviruses (5). In sharp contrast, however, virus-transfected fibroblasts are immortalized at a very low frequency (8, 16, 24). Also, human fibroblasts have been shown to be very resistant to immortalization by any other means *in vitro* and rarely develop tumors *in vivo*, suggesting that different, as yet unknown mechanisms operate in fibroblasts and epithelial cells in the process of immortalization. Since both KMST and SUSM cells are derived from fetal human fibroblasts, it is quite unlikely that the cessation of their indefinite division by the introduction of normal chromosome 7 was caused by tumor suppression or induction of terminal differentiation.

One may speculate that there is a novel control mechanism which determines the longevity of human cells in culture. At present, however, it would be premature to discuss further what cellular senescence or immortality has to do with differentiation and/or tumor suppression. Identification of the gene(s) on chromosome 7 that reverses the immortal phenotype of human fibroblasts should lead to a better understanding of these important biological phenomena.

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