Mouse A9 Cells Containing Single Human Chromosomes for Analysis of Genomic Imprinting

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Abstract

To develop a systematic in vitro approach for the study of genomic imprinting, we generated a new library of human/mouse A9 monochromosomal hybrids. We used whole cell fusion and microcell-mediated chromosome transfer to generate A9 hybrids containing a single, intact, bsr-tagged human chromosome derived from primary fibroblasts. A9 hybrids were identified that contained either human chromosome 1, 2, 4, 5, 7, 8, 10, 11, 15, 18, 20, or X. The parental origin of these chromosomes was determined by polymorphic analysis using microsatellite markers, and matched hybrids containing maternal and paternal chromosomes were identified for chromosomes 5, 10, 11 and 15. The imprinted gene KVLQT1 on human chromosome 11p15.5 was expressed exclusively from the maternal chromosome in A9 hybrids, and the parental-origin-specific expression patterns of several other imprinted genes were also maintained. This library of human monochromosomal hybrids is a valuable resource for the mapping and cloning of human genes and is a novel in vitro system for the screening of imprinted genes and for their functional analysis.

Key words: genomic imprinting; microcell-mediated chromosome transfer; in vitro assay system

1. Introduction

Genomic imprinting is a phenomenon where alleles of some mammalian genes are expressed differentially, depending on their parental origin. Imprinting has important consequences for development and tumorigenesis, as failure of correct imprinting induces abnormal embryogenesis, some inherited diseases, and is also associated with various cancers. Functional analyses have identified several features associated with imprinted genes and regions, including allele-specific DNA methylation, asynchronous replication timing and uniparental disomy (UPD) in genetic diseases. Approximately 20 imprinted genes have been identified in the mouse and human thus far. Examples of these include the H19 gene, which is expressed exclusively from the maternal allele, and the IGF2 and SNRPN genes, which are expressed from the paternal allele. However, the presence of both the maternal and paternal genomes in diploid cells is a significant barrier to the isolation of imprinted genes, and it is possible that many imprinted genes are yet to be identified. Any new systematic approach for the isolation of imprinted genes and analysis of the biological significance and mechanisms of genomic imprinting will need to address this problem.

Mouse A9 cells containing intact or fragmented human chromosomes are a useful source for the isolation of new DNA markers and the mapping of gene function to specific chromosomal regions. Microcell-mediated chromosome transfer from mouse A9/human monochromosomal hybrids to suitable recipient cells has been used to map genes involved in cellular aging, metastasis, DNA repair and tumor suppression. If the epigenetic status of transferred human chromosomes is maintained faithfully in A9 hybrids, for example parent-of-origin specific expression, methylation and replication timing, this should reflect the epigenetic status of chromosomal regions in the donor fibroblasts. A precedent for the maintenance of epigenetic status of human alleles in human/rodent hybrids is indicated by studies of X chromosome inactivation, where the inactivated human X chromosome remains inactive in human/mouse somatic cell hybrids. This system can be used to examine the inactivation, or escape from inactivation, of X-linked genes, whose expression reflects that in human cells.
In this study, we generated a new library of human/mouse A9 monochromosomal hybrids to establish an in vitro assay system for the analysis of genomic imprinting. This system is based on the separation of paternal and maternal chromosomes by construction of mouse A9 cells containing a single human chromosome, whose parental origin is known. Maintenance of the epigenetic status of imprinted genes in A9 hybrids will enable the comparison of allele-specific gene expression between these hybrids, without the need for the identification of an expressed polymorphism. This library, therefore, represents a powerful tool for the isolation of novel imprinted genes as well as the analysis of important features of genomic imprinting such as imprinted expression, and allele-specific epigenetic features such as methylation, replication timing and chromatin structure.

2. Materials and Methods

2.1. Cell lines and transfection

Primary culture of human dermal fibroblasts, isolated from a normal adult male, were established by standard methods. Mouse A9 cells were used as recipient cells for the introduction of normal human chromosomes by microcell fusion. Primary fibroblasts and mouse A9 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS, HyClone), 100 units/ml penicillin and 100 μg/ml streptomycin, in a humidified incubator at 37°C with 5% CO₂.

Human fibroblasts were transfected with circular pSV2bsr plasmid DNA (bsr) by the calcium phosphate precipitate method. Next, 2.5 x 10⁵ cells were seeded per 60-mm dish, followed by overnight incubation and transfection with 10 μg bsr plasmid per dish. The cells were incubated at 37°C for 4 hr, washed once with serum-free DMEM and treated with 1.5 ml of Hepes-buffered solution containing 15% fetal bovine serum (FBS, HyClone), 100 units/ml penicillin and 100 μg/ml streptomycin, in a humidified incubator at 37°C with 5% CO₂.

2.2. Cell hybridization

Direct transfer of bsr-tagged human chromosomes from BS-resistant fibroblast clones to mouse A9 cells was not successful, as human fibroblasts were resistant to microcell nuclei induction by colcemid treatment. Whole cell fusion of BS-resistant clones and mouse A9 cells was used to construct hybrid clones that were suitable microcell donors. BS-resistant fibroblasts and mouse A9 cells were seeded together, each at 1 x 10⁶ per 60-mm dish and were fused 24 hr later by the use of 5 ml of 41.7% (w/v) polyethylene glycol (PEG: MW 1000, Baker Chemical) containing 15% dimethyl sulfoxide (DMSO) for 1 min. The cells were then incubated with 25% PEG solution for 1 min and were then washed extensively with serum-free DMEM. The cells were maintained in nonselective medium for 24 hr and then plated into ten 100-mm dishes in selective medium containing 3 μg/ml of BS and 10⁻⁵ M ouabain. Hybrid clones were picked individually and used as donor cells for the transfer of bsr-tagged human chromosomes.

2.3. Microcell-mediated chromosome transfer

The method for microcell-mediated chromosome transfer was as described previously. Briefly, the isolated mouse/human hybrid clones were treated with 0.05 μg/ml of colcemid to induce the formation of micronuclei, which were then purified by cytochalasin B (10 μg/ml, Sigma Chemical) digestion and centrifugation. After centrifugation, the isolated microcells were resuspended in 12 ml of serum-free DMEM and filtered sequentially through 8-, 5-, and 3-μm polycarbonate filters (Nuclepore). The purified microcells were collected by centrifugation at 400 x g for 10 min and resuspended in 2 ml of serum-free DMEM containing 100 μg/ml of phytohemagglutinin (PHA, Difco). The microcells were attached to a monolayer of A9 cells at 37°C for 15 min. The microcells were fused with A9 cells in a 47% PEG solution for 1 min, followed by extensive washing in serum-free DMEM. The cells were maintained in nonselective medium for 24 hr, then trypsinized and split into three 90-mm-plates containing DMEM supplemented with 10% FBS and 3 μg/ml BS.

2.4. Chromosome analysis

Cells were prepared for chromosome analyses as described previously. Chromosomes were analyzed by Quinacrine plus Hoechst 33258 staining and the karyotypes of at least 20 well-banded metaphases were determined.

2.5. Fluorescence in situ hybridization (FISH)

FISH analysis to determine the integration site of the selectable marker DNA in the transferred human chromosome was carried out using the plasmid pSV2bsr as a probe. Briefly, fixed metaphase spreads were prepared for hybridization using standard methods. The pSV2bsr probe was labeled with biotin-16-dUTP by nick-translation (Boehringer Mannheim), purified by ethanol precipitation, dissolved in 20 μl of 100% formamide and denatured at 75°C for 10 min. Twenty microliters of the hybridization solution (bovine serum albumin [Boehringer Mannheim]: 10 x SSC: 50% dextran sulfate [Sigma], 1:2:2) and 20 μl of labeled probe DNA were combined in an Eppendorf tube, and then 20 μl of the mixture was dropped onto the denatured chromosomes, covered with paraffin, and incubated at 37°C.
for 15 hr in a humidified chamber. After hybridization, the slides were washed sequentially at 37°C in 50% formamide/2× SSC, 2× SSC, 1× SSC for 15 min each and once in 4× SSC for 5 min. The slides were incubated with anti-biotin Fab' alkaline phosphatase conjugate (1:100; Boehringer Mannheim) for 45 min at 37°C and hybridization signals were detected with 3-hydroxy-N-2'-biphenyl-2-naphthalenecarboxamide phosphate (HNPP; Boehringer Mannheim) for 45 min at 37°C and hybridization signals were detected with 3-hydroxy-N-2'-biphenyl-2-naphthalenecarboxamide phosphate (HNPP; Boehringer Mannheim). Chromosomes were counterstained with Quinacrine and Hoechst and observed with a NIKON fluorescence microscope.

### 2.6. Polymerase chain reaction (PCR)-based polymorphic analysis

PCR analysis of polymorphic microsatellites was used to determine the parental origin of human chromosomes in microcell hybrids. Genomic DNAs were purified from monochromosomal hybrids by standard procedures. PCR primer sequences for the amplification of microsatellites (Table 1) were obtained from the Genome Data Base and primers were 5' end-labeled using T4 polynucleotide kinase (Toyobo) and [γ-32P]dATP (Amersham). PCR amplification was performed in a volume of 10 μl with 100 ng of genomic DNA, 5 pmol each primer, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 1% Triton × 100, 50 μM of each dNTP and 0.25 units of Taq polymerase (Takara). Samples were processed through 30–35 cycles consisting of 94°C for 1 min, 55–60°C for 1 min and 72°C for 1 min followed by a final elongation for 4 min. The products were resolved by electrophoresis on 7 M urea-8% polyacrylamide gel and autoradiography.

### 2.7. Expression analysis in microcell hybrid clones

Genomic DNAs were purified by standard procedures. RNA was isolated from the microcell hybrids containing a single human chromosome 11 using a guanidine isothiocyanate-based method. DNase I (Takara)-treated total RNA was used a template for first strand cDNA synthesis in reactions containing oligo(dT)12–18, in the presence or absence of Superscript II reverse transcriptase (Gibco BRL) according to standard procedures. Amplification of genomic DNA and allelic specific expression of KVLQI1 was detected by using the primers LQTI11: 5'-AGA GAG GCC GGG CCC ACC ATT ACC CAG-3' and LQTI211: 5'-CAT GGC CCG GGT TAG GCT AA-3'. Samples were processed through 30 cycles of PCR consisting of 1 min at 94°C, 1 min at 60°C and 2 min at 72°C. The products were visualized by electrophoresis on 5% polyacrylamide gels.

To detect the L23MRP gene, the primers P2: 5'-GGT CTG CAG GGT TAG CTA-3' and P3: 5'-ATC CGG AAC TGC ACG GTG T-3' were used. Thermocycling conditions were denaturation for 1 min at 94°C, annealing for 30 sec at 57°C and extension for 40 sec at 72°C for 30 cycles. Biallelic expression of the L23MRP gene was detected by nested PCR as described previously.21 Briefly, the primers P1: 5'-CAT GCC GCG AAC TGC CAG TGT GGT TAG CTA-3' and P5: 5'-ATC GCC ACC TAG GCC ACC T-3' were used for RT-PCR, and amplification products were detected by PCR using the nested primers P1: 5'-CAT GCC GCG ACC TAG GCC ACC T-3' and P4: 5'-ATC GCC ACC TAG GCC ACC T-3'.

### 3. Results

#### 3.1. Isolation of microcell hybrid clones containing a human chromosome

The strategy we used to construct mouse A9 cells which contain a single human chromosome of known parental origin is outlined in Fig. 1. Human chromosomes were tagged by transfection of primary fibroblasts with the plasmid pSV2Icer. One thousand independent BS-resistant clones were isolated from five successive transfections and were expanded for whole cell hybridization. The isolated BS-resistant clones were pooled into 20 wells of 50 clones per well and cell fusion of each pool with mouse A9 cells was mediated by treatment with PEG fol-
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[Diagram showing the construction of mouse A9 cells containing a single human chromosome of known parental origin.]

lowed by selection with BS and ouabain. After 3 weeks, 70 human/mouse A9 hybrid clones were isolated and used as donor cells for human chromosome transfer. These hybrid clones each contained several human chromosomes in a mouse A9 cell background (data not shown). Transfer of the bsr-tagged human chromosome from these hybrid cells to mouse A9 cells was performed by microcell fusion. Seven hundred A9 microcell hybrid clones were isolated and karyotyped to examine the state of the introduced human chromosome, by double staining with Quinacrine plus Hoechst 33258 (Fig. 2). Forty-two A9 microcell hybrid clones contained either a single intact human chromosome 1 (1 clone), 2 (3 clones), 4 (2 clones), 5 (4 clones), 7 (2 clones), 8 (2 clones), 10 (3 clones), 11 (4 clones), 15 (2 clones), 18 (1 clone), 20 (1 clone) or X (1 clone), as summarized in Table 2. Under selective conditions the intact human chromosome was retained stably in each microcell hybrid clone (data not shown). The remaining microcell hybrid clones exhibited transferred chromosomes that were not intact, but were rearranged or fragmented.

The integration site of pSV2bsr on the human chromosome in these hybrid clones was confirmed directly by fluorescence in situ hybridization (FISH), as listed in Table 2. For example, the integration sites in A9(bsr11M)-1, A9(bsr11P)-1 and A9(bsr11P)-2 were mapped to human chromosome 11q14.3-22.2, q25 and q22.3-23.2, respectively (Fig. 2 and Table 2). This analysis indicates that clones were derived from independent pSV2bsr integration events, compared to duplicate clones generated during the expansion and cloning process, and confirms that these chromosomes contain the dominant selectable marker and are suitable for microcell transfer experiments.

3.2. Identification of parental origin of human chromosome in A9 microcell hybrid clones

PCR of human microsatellite markers (Table 1) was used to identify the parental origin of the human chromosome in A9 micr ocell hybrid clones, by comparison of genomic DNA derived from the microcell hybrids, the donor fibroblasts and parents of the donor (Fig. 3). Matching maternal and paternal pairs of human monochromosomal hybrids were identified for chromosomes 5 (Fig. 3A), 10, 11 (Fig. 3B) and 15, as summarized in Table 2. Of the four independent chromosome 5 hybrids, three were of maternal origin [A9(bsr5M)-1, 2 and 3] and one was of paternal origin [A9(bsr 5P)-1]. Of the three hybrid clones containing a single human chromosome 10, one clone was of maternal origin [A9(bsr10M)-1] and two clones were of paternal origin [A9(bsr10P)-1 and 2]. For chromosome 11, two hybrid clones were of maternal [A9(bsr11M)-1 and 2] and two of paternal origin [A9(bsr11P)-1 and 2]; and of the two chromosome 15 hybrids, one was of maternal [A9(bsr15M)-1], and the other of paternal origin [A9(bsr15P)-1]. The remaining microcell hybrids representing human chromosome 1, 2, 5, 7, 8, 18, 20 and X, contained chromosomes of a single parental origin, either paternal or maternal (Table 2).

3.3. Expression analysis of imprinted genes in microcell hybrid clones

We isolated microcell hybrid clones with matched copies of maternally and paternally derived chromosome 5, 10, 11 and 15. Of these, human chromosome 11 and 15 contain known regions that are subject to imprinting. 11p15.5 contains the imprinted genes H19,
IGF2, p57\textsuperscript{KIP2} and KVLQT1 among others, and imprinted regions implicated in familial paragangliomas exist at 11q13.1 and 11q22.3-q23.3. Chromosome 15 contains the imprinted regions responsible for Prader-Willi and Angelman syndromes at 15q11-11q13.

To confirm whether these hybrid clones could be utilized for analysis of genomic imprinting, we examined the expression of human KVLQT1, a potassium channel gene that causes the long QT syndrome (LQT) and is a maternally expressed, imprinted gene. The allelic expression pattern of KVLQT1 was analyzed by RT-PCR using a primers within exon 14, LQT111-LQT211. KVLQT1 was expressed only in hybrids that contained a maternally derived copy of chromosome 11, A9(bsr11M)-1 and -2 (Fig. 4). RT-PCR of the L23 (mitochondrial)-related protein (L23MRP), a gene that is not imprinted and lies 40 kb downstream of H19,\textsuperscript{21} demonstrated that it was expressed at equivalent levels in all of the human chromosome 11 microcell hybrid clones (Fig. 4). PCR amplification of genomic DNA using the same KVLQT1 and L23MRP primers indicated that this region was not deleted in paternal hybrids. This analysis demonstrated maternal specific expression of KVLQT1 in our human monochromosomal hybrids and indicated that imprinted expression was maintained following chromosome transfer from primary fibroblasts.

4. Discussion

We report here the construction of a library of mouse A9 clones containing a single, morphologically intact, human chromosome of known parental origin. Microcell hybrid clones which contain human chromosomes 1, 2, 4, 5, 7, 8, 10, 11, 15, 18, 20 or X were established by microcell-mediated chromosome transfer (Table 2). Human chromosomes in these microcell hybrid clones were stable in spite of continuous culture, and most of the clones retained bsr-tagged intact human chromosome under selective conditions. In combination with libraries we constructed previously,\textsuperscript{17} A9 hybrids for all human chromosomes, except for the Y chromosome and the inactive X chromosome, have now been generated. This system of library construction should enable the isolation of maternal and paternal copies of each human autosome. Human chromosomes in the library we report here have an integrated dominant selectable marker, and therefore can be introduced to other cell types using microcell transfer, for genetic analyses of cellular phenotypes.\textsuperscript{18,22} This library therefore serves as resource for the mapping and identification of genes that function during cellular aging involving telomerase regulation,\textsuperscript{12,23,24} metastasis,\textsuperscript{10} and DNA repair\textsuperscript{11} genes that are responsible for various inherited genetic defects\textsuperscript{25,26} or for the generation of transgenic mice with introduced human chromosomes and the establishment of animal models of human disease.\textsuperscript{27}
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Table 2. Library of A9 cells containing a single human chromosome tagged with the blasticidin S (BS)-resistance gene.

| Microcell hybrid clones | Human chromosome in microcell hybrids | Parental origin of chromosome | Integration site of pSV2 origin |
|-------------------------|---------------------------------------|------------------------------|--------------------------------|
| A9(bsr11M)-1            | #1                                   | Maternal                     | 1p36.3                         |
| A9(bsr10M)-1            | #2                                   | Maternal                     | 2p21-p23                       |
| A9(bsr9M)-2             | #3                                   | Maternal                     | 2q35                           |
| A9(bsr8P)-1             | #4                                   | Paternal                     | 4q13                           |
| A9(bsr8M)-1             | #5                                   | Maternal                     | 5q14                           |
| A9(bsr7M)-2             | #6                                   | Maternal                     | 5q14-5q15                      |
| A9(bsr7P)-1             | #7                                   | Paternal                     | 5q14                           |
| A9(bsr6P)-1             | #8                                   | Maternal                     | 6q21                           |
| A9(bsr6M)-1             | #9                                   | Paternal                     | 6q21-6q22                      |
| A9(bsr5M)-1             | #10                                  | Maternal                     | 10p15.3                        |
| A9(bsr5P)-1             | #11                                  | Maternal                     | 10q21.2-q21.3                  |
| A9(bsr4M)-1             | #12                                  | Maternal                     | 10q22                          |
| A9(bsr4P)-1             | #13                                  | Maternal                     | 11q14.3-q22.2                  |
| A9(bsr3M)-1             | #14                                  | Maternal                     | 11q14.3-q22.2                  |
| A9(bsr3P)-1             | #15                                  | Maternal                     | 11q15                          |
| A9(bsr2M)-1             | #16                                  | Maternal                     | 11q22-11q23                    |
| A9(bsr1M)-1             | #17                                  | Maternal                     | 11q23-11q24                    |
| A9(bsr0M)-1             | #18                                  | Maternal                     | 11q25                          |
| A9(bsr0P)-1             | #19                                  | Maternal                     | N.D.                           |
| A9(bsr9M)-1             | #20                                  | Maternal                     | 15q12.1-15q21                  |
| A9(bsr8M)-1             | #21                                  | Paternal                     | Xp11.2                         |

N.D., not determined.

A novel application for these A9 monochromosomal hybrids is the study of genomic imprinting. In contrast to our previous libraries, the parental origin of human chromosomes in these A9 hybrids could be determined by polymorphic analyses. For human chromosomes 5, 10, 11 and 15, A9 hybrids with maternally or paternally derived chromosomes were identified. If the epigenetic regulation of imprinted genes on human chromosomes was preserved in A9 cells following microcell transfer, these hybrids could provide an experimental system to study genomic imprinting. We examined the expression of KVLQ1T1, a maternally expressed imprinted gene, in A9 hybrids containing either maternal or paternal copies of human chromosome 11. KVLQ1T1 was expressed exclusively from microcell hybrid clones containing a maternally derived chromosome 11. This indicated that allele-specific expression of human KVLQ1T1 was maintained in mouse A9 cells. This was consistent with the analysis of other imprinted human genes in our monochromosomal hybrids. Maternal-specific expression of H19 on chromosome 11p15.5, and paternal specific expression of SNRPN and IPW on chromosome 15q11-q13, was also maintained in A9 cells. Analysis of the methylation of H19, SNRPN and IPW has also demonstrated that allele-specific methylation profiles were retained in A9 cells.

Furthermore, each parental human chromosome 11 was transferred independently into mouse near-diploid immortal fibroblasts (m5S) and the OTF9-63 and P19 embryonal carcinoma (EC) cell lines. The paternal allele of human H19 remained in a repressed state in m5S cells, but was de-repressed in both EC hybrids. The paternal H19 allele was demethylated in OTF9-63 cells, but the only alteration in P19 hybrids was de novo methy-
References

1. Surani, M. A., Barton, S. C., and Norris, M. L. 1986, Nuclear transplantation in the mouse: Heritable differences between parental genomes after activation of the embryonic genome. Cell, 45, 127–136.
2. McGrath, J. and Solter, D. 1984. Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell, 37, 179–183.
3. Cattanach, B. M. and Kirk, M. 1985, Differential activity of maternally and paternally derived chromosome regions in mice. Nature, 315, 496–498.
4. Solter, D. 1988. Differential imprinting and expression of maternal and paternal genomes, Annu. Rev. Genet., 22, 127–146.
5. Hall, J. G. 1990, Genomic imprinting: review and relevance to human disease, Am. J. Hum. Genet., 46, 857–873.
6. Neumann, B. and Barlow, D. P. 1996, Multiple roles for DNA methylation in gametic imprinting, Curr. Opin. Genet. Dev., 6, 159–163.
7. Kitsberg, D., Selig, S., Brandeis, M., Simon, I., Keshet, I., Driscoll, D. J., Nicholas, R. D., and Cedar, H. 1993, Allele-specific replication timing of imprinted gene regions, Nature, 364, 459–63.
8. Hurst, L. D. and McVean, G. T. 1997, Growth effects of uniparental disomies and the conflict theory of genomic imprinting, Trends Genet., 13, 436–443.
9. Uejima, H., Shinohara, T., Nakayama, Y., Kugoh, H., and Oshimura, M. 1998, Mapping a novel cellular senescence gene to human chromosome 2q37 via irradiation microcell-mediated chromosome transfer, Mol. Carcinogen., 22, 34–45.
10. Ichikawa, T., Nibei, N., Suzuki, H., Oshimura, M. et al. 1994, Suppression of metastasis of rat prostatic cancer by introducing human chromosome 8, Cancer Res., 54, 2299–2302.
11. Kurimasa, A., Nagata, Y., Shimizu, M., Emi, M., Nakamura, Y., and Oshimura, M. 1994, A human gene that restores the DNA-repair defect in SCID mice is located on 8p11.1-11.1. Hum. Genet., 93, 21–26.
12. Tanaka, H., Shimizu, M., Horikawa, I. et al. 1998, Evidence for a putative telomerase repressor gene on the 3p14.2-p21.1 region, Genes, Chrom. & Cancer, 23, 123–133.
13. Kahan, B. and DeMars, R. 1975, Localized derepression on the human inactive X chromosome in mouse-human cell hybrids, Proc. Natl. Acad. Sci. USA., 72, 1510–1514.
14. Migeon, B. R., Wolf, S. F., Maren, C., and Axelam, J. 1982, Derepression with decreased expression of the G6PD locus on the inactive X chromosome in normal human cells, Cell, 29, 595–600.
15. Brown, C. J. and Willard, H. F. 1994, The human X-inactivation centre is not required for maintenance of X-chromosome inactivation, Nature, 368, 154–156.
16. Izumi, M., Miyazawa, H., Kamakura, T., Yamaguchi, I., Endo, T., and Hanaoka, F. 1991, Blasticidin S-resistance gene (bsr): a novel selectable marker for mammalian cells, Exp. Cell. Res., 197, 229–33.
17. Koi, M., Shimizu, M., Morita, H., Yamada, H., and Oshimura, M. 1989, Construction of mouse A9 clones containing a single human chromosome tagged with neomycin-resistance gene via microcell fusion, Jpn. J. Cancer Res., 80, 413–418.
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18. Kugoh, H. M., Hashiba, H., Shimizu, M., and Oshimura, M. 1990, Suggestive evidence for functionally distinct, tumor-suppressor genes on chromosomes 1 and 11 for a human fibrosarcoma cell line, HT1080, Oncogene, 5, 1637–1644.

19. Kondoh, Y., Ono, T., Kagiyama, N. et al. 1995, Simultaneous visualization of Q-bands and FISH signals using a novel fluorochrome, Cytogenet. Cell Genet., 71, 96–98.

20. Lee M., P., Hu, R. J., Johnson L. A., and Feinberg, A. P. 1997, Human KVLQT1 gene shows tissue-specific imprinting and encompasses Beckwith-Wiedemann syndrome chromosomal rearrangements, Nat. Genet., 15, 181–185.

21. Tsang, P., Gilles, F., Yuan, L. et al. 1995, A novel L23-related gene 40 kb downstream of the imprinted H19 gene is biallelically expressed in mid-fetal and adult human tissues, Hum. Mol. Genet., 4, 1499–1507.

22. Goyette, M. C, Cho, K., Fasching, C. L. et al. 1992, Progression of colorectal cancer is associated with multiple tumor suppressor gene defects but inhibition of tumorigenicity is accomplished by correction of any single defect via chromosome transfer, Mol. Cell. Biol., 12, 1387–1395.

23. Sasaki, M., Honda, T., Yamada, H., Wake, N., Barrett, J. C., and Oshimura, M. 1994, Evidence for multiple pathways to cellular senescence, Cancer Res., 54, 6090–6093.

24. Ohmura, H., Tahara, H., Suzuki, M. et al. 1995, Restoration of the cellular senescence program and repression of telomerase by human chromosome 3, Jpn. J. Cancer Res., 86, 899–904.

25. Ishizaki, K., Oshimura, M., Sasaki, M. S., Nakamura, Y., and Ikenaga, M. 1990, Human chromosome 9 can complement UV sensitivity of xeroderma pigmentosum group A cells, Mutat. Res., 235, 209–15.

26. Jongmans, W., Verhaegh, G. W., Jaspers, N. G. et al. 1996, The defect in the AT-like hamster cell mutants is complemented by mouse chromosome 9 but not by any of the human chromosomes, Mutat. Res., 364, 91–102.

27. Tomizuka, K., Yoshida, H., Uejima, H. et al. 1997, Functional expression and germline transmission of a human chromosome fragment in chimaeric mice, Nat. Genet., 16, 133–143.

28. Zhang, Y. and Tycko, B. 1992, Monoallelic expression of the human H19 gene, Nat. Genet., 1, 40–44.

29. Rainier, S., Johnson, L. A., Dobry, C. J., Ping, A. J., Grundy, P. E., and Feinberg, A. P. 1993, Relaxation of imprinted genes in human cancer, Nature, 362, 747–749.

30. Glenn, C. C., Porter, K. A., Jong, M. T. C., Nicholls, R. D., and Driscoll, D. J. 1993, Functional imprinting and epigenetic modification of the human SNRPN gene, Hum. Mol. Genet., 2, 2001–2005.

31. Nakao, M., Sutschl, J. S., Dutschki, B., Mutirangura, A., Ledbetter, D. H., and Beaudet, A. L. 1994, Imprinting analysis of three genes in the Prader-Willi/Angelman region: SNRPN, E6-associated protein, and PAR-2 (D15S225E), Hum. Mol. Genet., 3, 309–315.

32. Reed, M. L. and Leff, S. E. 1994, Maternal imprinting of human SNRPN, a gene deleted in Prader-Willi syndrome, Nature Genet., 6, 163–167.

33. Wevrick, R., Kerns, J. A., and Francke, U. 1994, Identification of a novel paternally expressed gene in the Prader-Willi syndrome region, Hum. Mol. Genet., 3, 1877–1882.

34. Meguro, M., Mitsuya, K., Sui, H. et al. 1997, Evidence for uniparental, paternal expression of the human GABAA receptor subunit genes, using microcell-mediated chromosome transfer, Hum. Mol. Genet., 6, 2127–2133.

35. Mitsuya, K., Meguro, M., Sui, H. et al. 1998, Epigenetic reprogramming of the human H19 gene in mouse embryonic cells does not erase the primary parental imprint, Genes Cells, 3, 245–255.

36. Toder, R., Wilcox, S. A., Smithwick, M., and Graves, J. A. 1996, The human/mouse imprinted genes IGF2, H19, SNRPN and ZNF127 map to two conserved autosomal clusters in a marsupial, Chromosome Res., 4, 295–300.

37. Kuroiwa, Y., Shinohara, T., Notsu, T. et al. 1998, Efficient modification of a human chromosome by telomere-directed truncation in high homologous recombination-proficient chicken DT40 cells, Nucleic Acids Res., 26, 3447–3448.