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Comparison of Established Cell Lines at Different Passages by Karyotype and Comparative Genomic Hybridization

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Two established cancer cell lines, MCF-7 and Ishikawa, were both obtained directly from a cell repository and through another laboratory. The karyotypes from the two MCF-7 cell lines had up to 83 chromosomes and similarities for chromosomal gain and structural abnormalities. The two Ishikawa cell lines had up to 60 chromosomes with only a missing X as the common chromosome abnormality. CGH studies were performed by co-hybridizing the two Ishikawa or MCF-7 cell lines to normal metaphases. The differences seen between the two MCF-7 cell cultures reflect changes due to passage number and culture conditions. For Ishikawa, DNA polymorphic data and mutation studies suggest that the two cell lines are not derived from the same established tumor cell line. Our study shows the utilization of CGH in comparing cell lines originating from the same specimen. Our study also demonstrates the necessity for periodically evaluating cell lines to confirm their origin.

KEY WORDS: Comparative genomic hybridization; established cell lines; karyotype; chromosome abnormalities.

INTRODUCTION

Culturing of primary tumors for use in studying cancer cells can be difficult to establish. Utilization of commercially available transformed immortal cell lines that are well characterized by cytogenetics and biochemical markers allows comparison of results between laboratories. However, as these cell lines tend to be maintained in culture over a long period of time, mutations can occur that may change characteristics that have initially been identified at earlier passages.

Comparative genomic hybridization (CGH) can be a useful technique for detecting chromosomal duplications and deletions [1]. This technique has been extremely useful in identifying complex rearrangements occurring in solid tumors [2]. A

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disadvantage of the technique is that it cannot adequately evaluate heterogenous cell populations [3], which can be a problem with cell lines that are cultured for long periods of time.

We studied two cultures from two established cancer cell lines, MCF-7 (breast cancer), and Ishikawa (endometrial tumor), which were maintained in different laboratories and subcultured over 100 times. The karyotypes of the two MCF-7 cultures and the two Ishikawa cultures were different. Because of extensive rearrangement, there were many unidentifiable markers. In order to determine how similar or divergent the parallel cultures were, CGH was performed by co-hybridizing DNA from the two MCF-7 or two Ishikawa cultures. DNA instability studies and DNA polymorphisms were also analyzed in the four cultured cell lines.

MATERIALS AND METHODS

Cell Cultures

MCF-7 was derived from breast tumor: MCF-7-ATCC was obtained from American Type Culture Collection at passage 149 and passaged an additional 16 times in Dulbecco's minimal essential medium (DMEM) with 10% FBS and 5 μM insulin; MCF-7-RIDC had been obtained from the laboratory of Dr. Billy W. Day at the University of Pittsburgh after an unknown number of passages and then subcultured in DMEM plus 10% FBS an additional 95 times. MCF-7-RIDC has previously been shown to have a baseline HPRT mutation frequency 10-fold higher than MCF-7-ATCC [4]. Ishikawa was derived from an endometrial tumor: Ishikawa-ECCC obtained from the European Collection of Cell Cultures at passage 3+ was ER positive, and was subcultured in DMEM plus 15% FBS an additional 130 times; Ishikawa-UNC had been maintained in another laboratory from Dr. Bruce A. Lessey of the University of North Carolina at Chapel Hill after an unknown number of passages, was ER negative, and was subcultured in DMEM plus 10% FBS an additional 132 times. Ishikawa-ECCC had a baseline HPRT mutation frequency 1400-fold higher than Ishikawa-UNC [4].

Cytogenetics

Cultures were split 1:2 using media utilized by corresponding laboratories and harvested 2 days later using routine cytogenetic techniques. Cells were Giemsa-banded and 9–15 cells were karyotyped per cell line. The karyotype designation for each cell line was a composite since all cells analyzed were different but had some common chromosomal abnormalities.

CGH

DNA was extracted from each cell line. Each pair of MCF-7 or Ishikawa cell lines was labeled with spectrum red or spectrum green nick translation reagent kit (Vysis, Inc, Downers Grove, IL) following manufacturer's protocol. DNA from the paired cell lines were co-hybridized for 3 days to normal metaphase chromosomes which had been denatured. Cells were analyzed using the CGH program on the

Table 1. Primers sequences and PCR conditions for STS markers used for polymorphism analysis

Locus and type of sequence amplified	Primers sequence	Ta	Product size ranges	Maximum heterozygosity
D17S855(CA)n	F 5′-GGATGGCCTTTTAGAAAGTGG-3′ R 5′- ACACAGACTTGTCCTACTGCC-3′	60–58	143–155 bp	0.8220
D17S934(CA)n	F 5′-TCTGAATGGCCCTTGG-3′ R 5′-TCCTATCTGAGGTGGGGT-3′	58–55	186–190 bp	0.8412
D17S787(CA)n	F 5′-TGGGCTCAACTATATGAACC-3′ R 5′-TTGATACCTTTTTGAAGGGG-3′	58-56	138–166 bp	0.8169

Ta = Primer annealing Temperature; bp = base pair length; F = forward; R = reverse.

Applied Imaging system. A minimum of 30 cells of good hybridization quality were analyzed for green and red fluorescence ratio.

DNA Polymorphism Analysis at STS Markers Loci

Primers for Microsatellite Markers. The oligonucleotide primers for microsatellite markers (D17S855-D17S934-D17S787) were selected from GDB database (http://gdbwww.gdb.org/). The sequence and details of each primer pair are listed in Table 1.

PCR and STS Marker Polymorphism Analysis. DNA was amplified by PCR in a 12.5 μl reaction mixture. The PCR mix included 12.5 picomole of each primer (forward and reverse), 200 nM of dNTP with 0.5 unit of *Taq* polymerase enzyme, 1 mM of $MgCl_2$ and 1× PCR buffer. Thermal cycling was performed on a PTC-100 Programmable Thermal Controller (MJ Research Inc.), using the following conditions: initial denaturation at 94°C for 3 min, 30 cycles at 94°C for 30 s, 60–55°C, 72°C for 30 s and a final extension of 72°C for 5 min. The amplified products were mixed with SSLP dye (90% formamide, 0.05% bromophenol blue, 0.05% xylene cyanole, 1 mM EDTA), and electrophoresed through 12% of polyacrylamide gel (29:1 acrylamide: Bis acrylamide). The gels were silver stained and photographed.

RESULTS

Karyotype similarities between the two MCF-7 cell lines included +2, +2, +3, add(3)(p25), +4, +5, +6,del(6)(q21), +del(6)(q21), +7, +8, +9, +10, +12, +13, +14, +15, +17, +19, +20,add(22)(q13) (Figs. 1 and 2). MCF-7-ATCC was in culture longer than MCF-7-RIDC, which would explain its more complex karyotype. Differences in karyotypes identified by CGH included an extra chromosome 9 and duplications for 1q, 5p, 5q, and 12p in the RIDC line (Fig. 3). The other changes would be related to different marker chromosomes, as well as mosaicism for other chromosome abnormalities not identified in the composite karyotype.

The only similarity between the karyotypes of the two Ishikawa cell lines was a missing X chromosome (Figs. 4 and 5). Differences in the karyotype identified by CGH included an extra chromosome 2, duplications of 4q, 6p, 8p, 9p, 12p and 13q for Ishikawa-UNC, and 14q and 21q duplications for Ishikawa-ECCC. DNA

Fig. 1. The composite karyotype for MCF-7-ATCC was 64-83,X,add(X)(p22.3), + 2, + 2, + 3,add(3)(p25), + 4, + 5, + 6,del(6)(q21), + del(6)(q21), + 7, + 8, + 9, + 10,add(11)(p15), + 12, + 13, + 14, + 15, + 17, 17, + 19, + 20,add(22)(q13),6-14 mar,2-6 min[cp9].

microsatellite analysis showed similar alleles for MCF-7 cell lines, but different alleles for the two Ishikawa cell lines (Fig. 6).

DISCUSSION

Normally, CGH is performed by co-hybridization of a tumor cell line with a normal cell line. Our study showed that it is possible to co-hybridize two tumor cell lines to determine differences between the two karyotypes. These differences would indicate divergent changes the cell lines have gone through by serial passaging in different laboratories and different media conditions. Further studies could include spectral karyotyping or specific FISH chromosome paints for some of these areas identified by CGH to help further characterize the karyotypes.

The MCF-7 cell lines showed some karyotype differences, most likely reflecting the difference in cell passage numbers and maintenance in different laboratories. Even in characterized cell lines, because these cells are continually evolving, there is heterogeneity between cells within the same culture [5], which was evident in our karyotypes. While there were similarities between the two cell lines, there were also non-clonal abnormalities within each cell line. This divergence in karyotype was evident by CGH co-hybridization of the two cell lines together. Studies in Chinese hamster cells have shown that structurally abnormal chromosomes increase more rapidly in aneuploid cells than diploid or near-diploid cell lines [6]. Initial heterogeneity in cell lines would show divergent chromosomal changes.

Fig. 2. The composite karyotype for MCF7-RIDC was 43-83,X,add(X)(q26), + 1, + 2, + 2, + 3,add(3)(p25), + 4, + 4, + 5, + 5, + 6,del(6)(q21), + del(6)(q21), + 7,dup(7)(p13p15),dup(7)(p13p15), + 8, + 9, + 9, i(9)(q10), + 10, + 10, + 11,del(11)(p13), + 12,del(12)(p11.2), + 13, + 14, + 14, + 15, + 15, + 16, + 16, + 17, del(17) (p13), + 18, + 19,der(19)t(12;19)(q13;q13.3), + 20,-21,-22, add(22)(q13), + 6-17 mar[cp14].

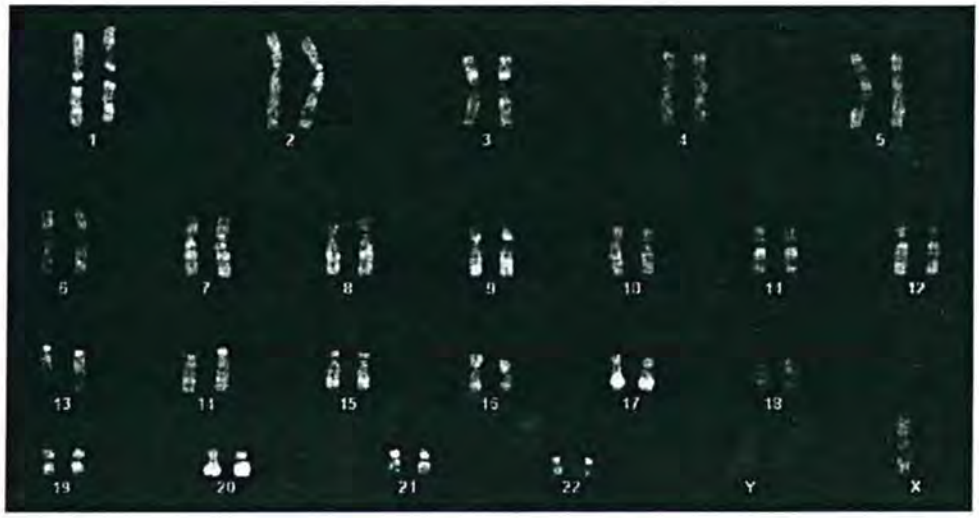

Fig. 3. CGH between MCF-7 cell lines.

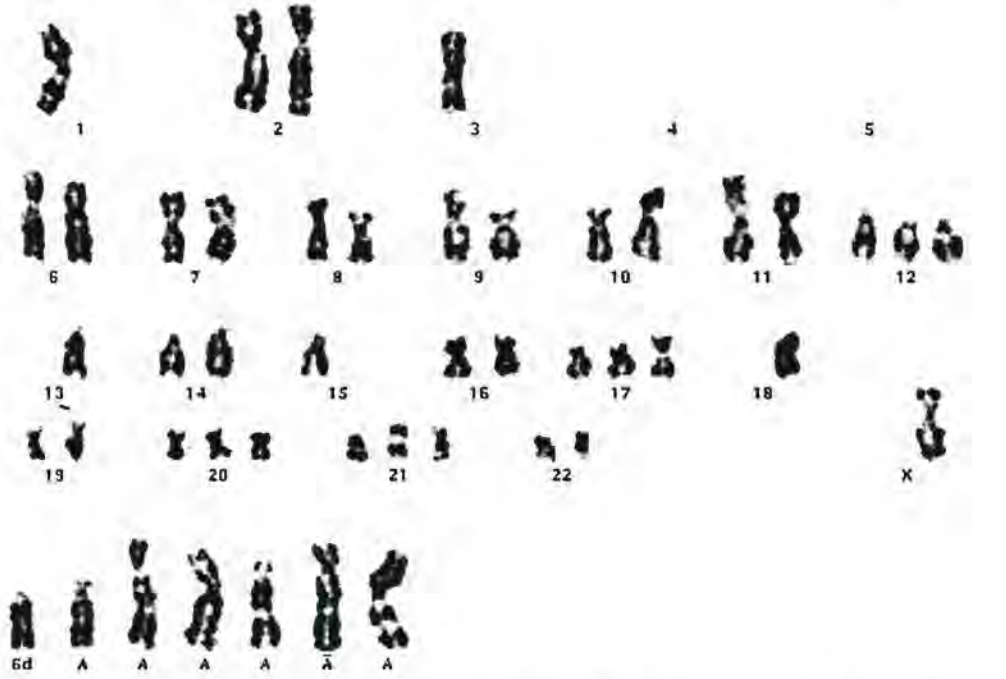

Fig. 4. The composite karyotype for Ishikawa-ECCC was 42–53,X,-X,+2,-4, del(6) (q23), add(7)(q36),-8,-9,add(11)(p15),del(12)(p11.2),+del(12)(p11.2), add(13)(q34),i(14)(q10),-15,add(21)(q22),+6 mar[cp12].

A recent report evaluated the karyotypes of three cell lines of MCF-7 obtained from different laboratories by CGH. There were differences by CGH as well as the proliferative response to 17-β-estradiol [7]. Multiple cell lines of MCF-7 were karyotyped, all showing multiple aneuploidy and structural abnormalities. The heterogeneity of the cultures reflected genetic instability of these cell lines, which may be related to the aggressiveness of this tumor [8]. The same cell lines karyotyped 6 years later had different karyotypes but still had some similar markers [8].

While the Ishikawa-UNC cell line was passaged longer than Ishikawa-ECCC, the karyotype was not as complex. The Ishikawa karyotypes, while not as complex as the MCF-7 cell lines, showed no similarities other than a missing X chromosome. The baseline mutation frequency was very different and microsatellite analysis showed different alleles between the two Ishikawa cell lines, suggesting different origin of the two cell lines. It is possible that these cell lines, although both called Ishikawa, may be different origins, one that was ER negative, the other ER positive. It is also possible that one of these cell lines became mislabeled, or contaminated with another cell line present in the same laboratory [9]. It is important when working with established cell lines that all cultures are handled separately for each cell line and that cell lines be checked periodically to verify their origin.

Our laboratory recently karyotyped a cell culture from another laboratory that was using an established human cell line for their research. The karyotype revealed that the cell line was rat, not human. The other laboratory also had rat cell lines that were being maintained along with the human cell line, suggesting contamination of cell lines or mislabeling of flasks.

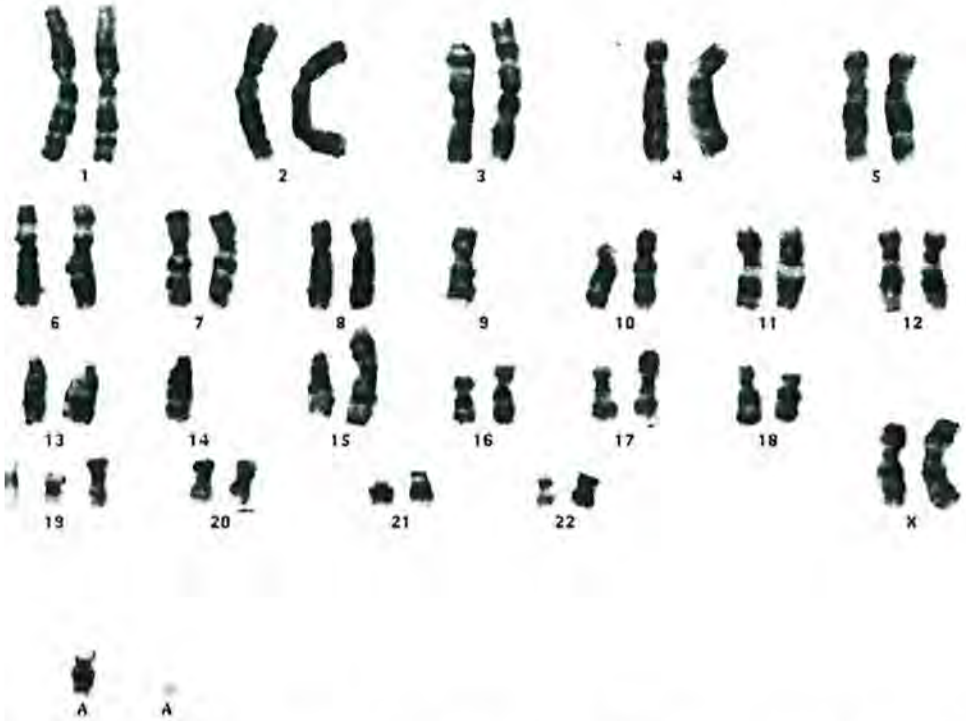

Fig. 5. The composite karyotype for ISHIKAWA-UNC is 44-60,X,-X,add(3)(p26),+4,+6, +7,+8,+ 13,add(17)(p13),+19,+20,-22,+4 mar[cp10].

This may be the first report of using CGH from the same cell lines to make comparisons between the two rather than co-hybridizing each separately with normal cell lines and then comparing the results. The latter has the advantage of being able to give information specific for each cell line as to copy number of chromosomes and regions. By co-hybridizing the two cell lines, it was possible to readily distinguish the differences between them, although not as easy to interpret as to specific gains and losses for each of the cell lines due to non-clonal changes.

This study shows the utility of CGH for comparing karyotypic differences between cell cultures from the same established cell line. It emphasizes the importance of confirming the origin of cultured cells used for research, especially when obtained from a source other than a cell repository. It also illustrates changes that a cell line can undergo with multiple passages, which has the potential of changing some of the characteristics of the cell line from earlier passages. Confirmation of cell line origin and its characteristics is imperative before using cell lines in experiments for reliability in reporting experimental results to other researchers.

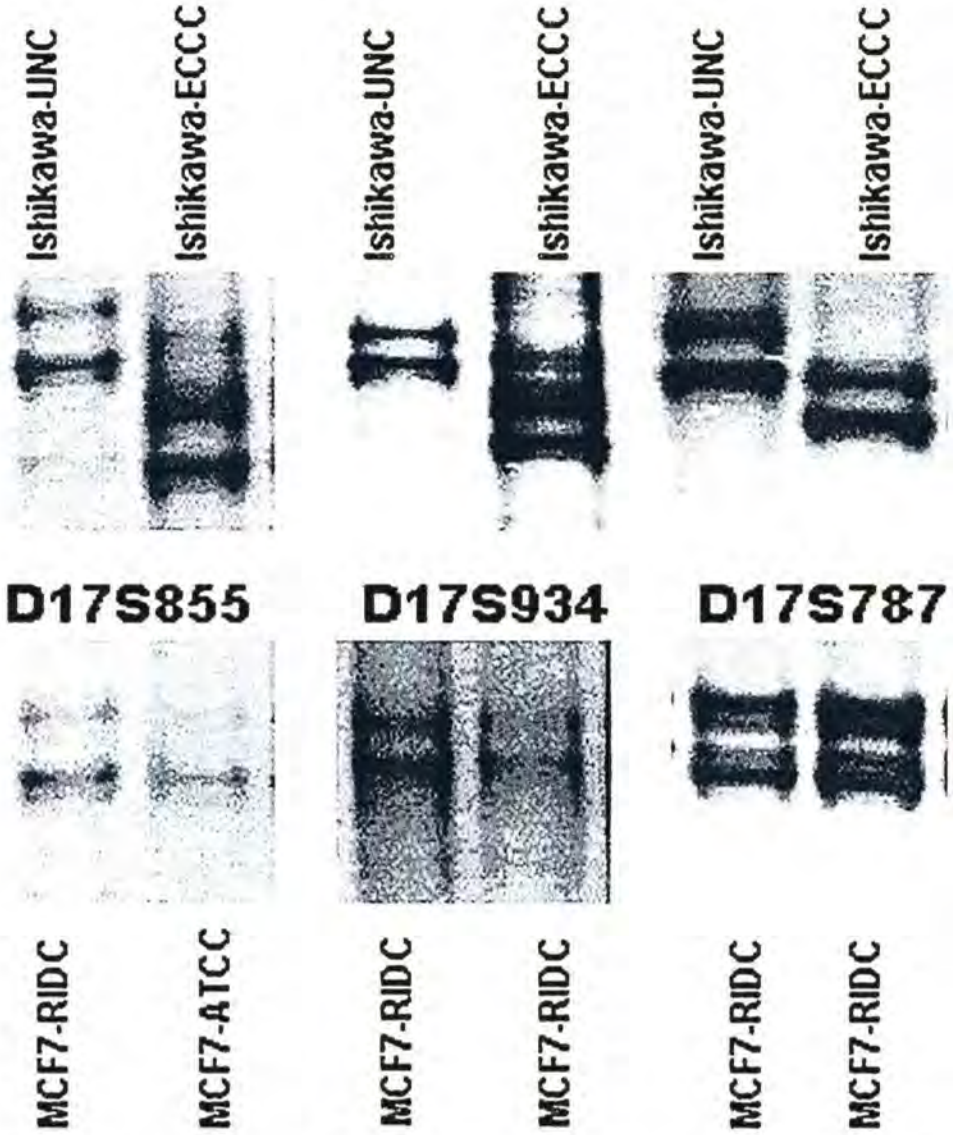

Fig. 6. DNA microsatellite analysis (D17S855-D17S934-D17S787) of two MCF-7 and two Ishikawa cell lines.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

REFERENCES

1. Lapierre, J. M., Cacheux, V., Da Silva, F., Collot, N., Hervy, N., Wiss, J., and Tachdjian, G. (1998) *Ann. Genet.* **41**:56–62.
2. Kallioniemi, A., Kallioniemi, O., Sudar, D., Rutovitz, D., Gray, J. W., Waldman, F., and Pinkel, D. (1992) *Science* **258**:818–821.

3. Wang, N. (2002) *Am J. Med. Genet.* **115**:118–124.
4. Joshi, N. and Grant, S. G. (2002) *Am. J. Hum. Genet.* **71**(suppl) 251.
5. Wolman, S. R. and Heppner, G. H. (1992) *J. Natl. Cancer Inst.* **84**:469–470.
6. Fabarius, A., Hehlmann, R., and Duesberg, P. H. (2003) *Cancer Genet. Cytogenet.* **143**:59–72.
7. Jones, C., Payne, J., Wells, D., Delhanty, J. D. A., Lakhani, S. R., and Kortenkamp, A. (2000) *Cancer Genet. Cytogenet.* **117**:153–158.
8. Whang-Peng, J., Lee, E. C., Kao-Shan, C.-S., Seibert, K., and Lippman, M. (1983) *J. Natl. Cancer Inst.* **71**:687–695.
9. Gold, M. (1986) *A Conspiracy of Cells: One Woman's Immortal Legacy and the Medical Scandal it Caused.* State University of New York Press, Albany.