Isolation and Characterization of a Highly Malignant Variant of the SW480 Human Colon Cancer Cell Line

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ABSTRACT

We found that the human colon cancer cell line SW480 consists of two distinct subpopulations which we have designated E-type (epithelial) and R-type (round). Pure cultures of each type were obtained by subcloning, and both have maintained their characteristic phenotypes for at least 1 year (40 passages). E-type cells are the major (>98%) type in the parental SW480 cell line. They form flat epithelial-like colonies. In contrast, R-type cells, which constitute a minor fraction (<2%) of the parental cell line, have a rounded shape and grow in clusters of piled-up cells. Compared to E-type cells or the parental SW480 cells, isolated R-type cells display decreased doubling time, loss of contact inhibition, less adhesiveness to culture plates, higher anchorage-independent growth in soft agar, and a much more aneuploid karyotype. When injected s.c. into nude mice, R-type cells produce much larger tumors within the same period of time than E-type cells, and the tumors are less differentiated than those produced by the E-type cells. Cell fusion experiments between R-type and E-type cells revealed that the R-type phenotype is dominant, and the results suggest that this is due to one or a few genetic changes. Taken together, these findings suggest that the R-type cells represent a more malignant variant of the E-type cells. They may be useful, therefore, for studying mechanisms involved in tumor progression.

INTRODUCTION

Colorectal cancer is one of the most frequent malignant tumors and is the second leading cause of cancer deaths in the United States. Extensive epidemiological and clinical studies suggest that colon tumors arise through a multistage process as a result of the interaction between multiple factors (1, 2). Evidence obtained from molecular biological studies indicates that this multistage process is associated with the progressive acquisition of mutations in oncogenes and putative tumor suppressor genes (3, 4). However, the molecular mechanisms responsible for this multistage process remain to be determined.

Cell lines established from human colorectal carcinomas provide useful tools to study the complex process of colon carcinogenesis. Among a number of established colon cancer cell lines, the SW480 line (ATCC CCL 228) is one of the best characterized lines. This cell line was isolated from a Duke’s B colon adenocarcinoma resected from a 50-year-old male patient by Leibovitz et al. in 1976 (5). In addition to the basic characterization described in the original paper, numerous biochemical and genetic properties of this cell line have been reported. These include the production of plasminogen activators and inhibitors (6), transforming growth factor β and its receptor (7), platelet-derived growth factor (8), the ability to bind to collagen via non-integrin-like receptors (9), point mutation in codon 12 of the c-K-ras gene (10), c-myc amplification (11), a point mutation in p53 (12), deletion of chromosome 18 (the DCC gene) (13), and loss of the APC gene on chromosome 5 (14).

During the course of experiments in our laboratory using the SW480 cell line, we noticed the existence of a distinct subpopulation of cells that had a round rather than epithelial morphology. In this paper we describe the isolation and characterization of this subtype and present evidence that it represents a more malignant variant of the parental SW480 cells. This variant may be useful for studies of the process of progression during colon carcinogenesis.

MATERIALS AND METHODS

Cell Culture. The SW480 cell line was obtained from the American Type Culture Collection. The cells were grown in DMEM* (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS (HyClone Laboratories, Logan, UT), penicillin (25 units ml⁻¹), streptomycin (25 μg ml⁻¹). Cultures were maintained in a humidified incubator at 37°C with 5% CO₂. For cell cloning, SW480 cells were plated onto 15-cm plates at a density of 5 × 10⁴ cells/plate. The morphology of individual colonies was examined under a phase contrast microscope (Phase Contrast-2; Nikon, Tokyo, Japan) 7 days later, and two distinct types of colonies were identified. Several independent colonies of each cell type were picked, and clonal cultures were established and expanded.

Growth Studies in Monolayer Cultures. Growth curves were performed by seeding a series of replicate plates with 5 × 10⁴ cells/35-mm plate in DMEM containing 10% FCS. The following day, cells from one set of plates were trypsinized and counted. This point was designated as day 0. The remaining cultures were then grown, with fresh medium changes every 2 days. Cell counts were performed in triplicate at subsequent 2-day intervals, using a Coulter counter (Model ZF; Coulter Electronics, Inc., Hialeah, FL).

Soft Agar Assay. To assay anchorage-independent growth in soft agar, 2 × 10⁴ cells were suspended in 2 ml of 0.3% agar (Agar Noble; Difco Laboratories, Detroit, MI) in DMEM containing 10% FCS and overlaid above a layer of 3 ml of 0.5% agar in the same medium, in 35-mm dishes. Cells were then overlaid with 2 ml of 0.3% agar in the same medium, once a week. After 21 days, colonies were stained with p-iodonitrotetrazolium violet (INT Solution; Sigma Diagnostics, St. Louis, MO) for 2 days at 37°C in an incubator with 5% CO₂. Colonies larger than 0.25 mm in diameter were then counted.

Tumorigenicity Assay in Nude Mice. Exponentially growing cells were trypsinized, washed with PBS, and reseeded in PBS at a concentration of 5 × 10⁵ cells/ml. Cells (5 × 10⁶) were injected s.c. into four sites of 4-week-old female BALB/c nu/nu athymic mice (Harlan Sprague Dawley). These mice were examined for tumor growth by palpation twice a week. After 25 days, all of the mice were sacrificed, and the tumors were weighed. Histological examination was performed using hematoxylin-eosin staining.

Cell Fusion Procedure. Neomycin- or hygromycin-resistant E8 and R2 cell lines were obtained by transfection of these cells with the plasmids pMV7 (15) or PRSV1.1 (16), which contain the neomycin- or hygromycin-resistant genes, respectively. The transfection using Lipofectin (Bethesda Research Laboratories, Life Technologies, Inc.) was

*The abbreviations used are: DMEM, Dulbecco's modified Eagle's minimal essential medium; FCS, fetal calf serum; PBS, phosphate-buffered saline.
performed according to the manufacturer's protocol. After selection with 1 mg/ml of G418 (Gibco Laboratories) (to obtain neo⁺ clones) or hygromycin B (Boehringer Mannheim, Mannheim, Germany) (to obtain hygro⁺ clones) several resistant clones were obtained. Clones E8 neo-10, E8 hygro-1, and R2 neo-3 were used for the cell fusion experiments. Cell fusion was performed according to the method of Davidson and Gerald (17). G418-resistant cells (1.5 × 10⁶) and the same number of hygromycin-resistant cells were plated onto 35-mm plates (total cells per plate, 3 × 10⁶). On the following day, the cells were washed twice with PBS and treated with 4 ml of polyethylene glycol (molecular weight, 1000; Sigma) (50% w/v in DMEM) for 30 s. The cells were then washed immediately with DMEM three times and cultured in DMEM containing 10% FCS, at 37°C overnight. The cells were trypsinized the following day, expanded into four 10-cm plates, and cultured for 2 days. Hybrids were selected in medium containing 1 mg/ml of G418 and 1 mg/ml hygromycin B. After 14 days, the total number of colonies that were both G418- and hygromycin-resistant was counted, and their morphologies were examined using phase contrast microscopy. Individual hybrid clones were picked, expanded, and used for further analysis.

Karyotypic Analysis. Metaphase spreads were prepared from exponentially growing cells that had been treated with 0.1 mg/ml of Colcemid (Gibco Laboratories) for 4 h. The cells were then trypsinized, collected, and further treated with 0.075 M KCl for 20 min, fixed with acetic acid:methanol (1:3) 3 times, and trypsin-Giemsa banded. Karyotypes were prepared and then analyzed using the standard International System for Human Cytogenetic Nomenclature (18).

Flow Cytometric Analysis. Exponentially growing cells were trypsinized, washed twice with cold PBS, and fixed with 70% cold ethanol. The DNA content of fixed cells was determined by flow cytometry using a FACS 440 instrument (Becton-Dickinson, Mountain View, CA).

RESULTS

Cloning of Two Sublines from SW480 Cells. The majority of SW480 cells attached to plastic plates after trypsinization and plating and formed typical epithelial colonies composed of cells with a cuboidal shape (Fig. 1, A and B). Close microscopic examination, however, revealed that a small number of colonies consisted of cells that had a rounded and refractile morphology and were piled up on each other and attached loosely to the plates. To determine whether these colonies represented a distinct subtype we isolated a series of individual clones from the SW480 parental cell line.

We obtained twelve independent colonies of SW480 cells that had the round morphology, which we designated round (R)-type. Most of these subclones grew well and maintained their round morphology during serial passage. Five independent R-type clones (R2, R5, R6, R9, and R11) were examined in detail. They all had similar phenotypes, i.e., rounded cells that had poor adhesiveness to the culture plates, and a tendency to pile up into clusters. When R-type cells reached about 50% confluency, they began to detach from the plate, and eventually the majority floated off the plates. The detached cells were no longer viable since they failed to replate and grow (data not

Fig. 1. Morphologies of the SW480 cell line and its subclones R2 and E8. The parental SW480 cells are shown at × 40 (A) and at × 80 (B). The R2 clone is shown at × 80 (C) and ×200 (D). The E8 clone is shown at × 80 (E) and ×200 (F).
Table 1 Summary of properties of E- and R-type cells

<table>
<thead>
<tr>
<th>Property</th>
<th>E-type</th>
<th>R-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth in culture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>Epithelial-like cuboidal</td>
<td>Rounded</td>
</tr>
<tr>
<td>Plate adheriveness</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Pattern of growth</td>
<td>Monolayer</td>
<td>Piled up</td>
</tr>
<tr>
<td>Doubling time</td>
<td>32.6 h</td>
<td>15.6 h</td>
</tr>
<tr>
<td>Growth in soft agar</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Tumorigenicity in nude mice</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Glandular formation in the nude mouse tumors</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chromosome number</td>
<td>58</td>
<td>94-99</td>
</tr>
<tr>
<td>Genetic changes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K-ras mutation</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c-myc amplification</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>p53 mutation</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

a Doubling times were calculated from the growth curves presented in Fig. 3.

b The point mutation in codon 12 of the K-ras gene was analyzed by the method of Jiang et al. (23).

c An approximately 10-fold amplification of c-myc was confirmed by Southern blot hybridization (24) using a 1.4-kilobase Clal- EcoRI fragment of pMC413RC (25) as a probe.

d The point mutation in exon 9 of the p53 gene was confirmed by single-strand conformation polymorphism analysis (26).

A representative clone of R-type cells, R2, was used for further analysis, and its morphology is shown in Fig. 1 (C and D). A number of typical epithelial colonies, designated E-type, were also obtained. A representative clone of this type, E8, was used for further analysis, and its morphology is shown in Fig. 1 (E and F). Both E8 and R2 cells have stably maintained their morphologies for over 1 year and 40 serial passages. The R-type cells in the parental population of SW480 cells constituted about 1.5% of the total cell population.

Identification of the Origin of E- and R-Type Cells. Because of the striking differences in the morphology of the E- and R-type cells, it was important to establish the origin of the R-type subpopulation. To do so, we analyzed three previously described genetic changes seen in the SW480 cell line, namely, a point mutation in codon 12 of c-K-ras (10), amplification of c-myc (11), and a point mutation in exon 9 of the p53 gene (12). We found that all three genetic changes were detected in the E8, R2, and parental SW480 cells (Table 1 and data not shown here). These findings suggest that the E- and R-type cells have a common origin. Additional evidence was provided by the karyotypic analysis of E8 and R2 cells (described below and in Table 6). Therefore, R2 cells are not simply a tissue culture contaminant in the SW480 cell line.

Growth Rate of E- and R-Type Cells in Culture. To further characterize the growth properties of these cells, we first compared the rates of growth of the two sublines in monolayer culture. Growth curves indicated that R2 cells grew faster than either the E8 clone or the parental SW480 cell line (Fig. 2). The doubling times of R2, E8, and SW480 cells, calculated from their respective growth curves during logarithmic growth, were 15.6, 31.6, and 28.2 h, respectively. Since extensive cell detachment occurred when the R2 cells reached about 50% confluence, the growth curve of the R2 cells after that point is indicated by a broken line in Fig. 2.

Growth of E- and R-Type Cells in Soft Agar. When cultured in 0.3% soft agar, both E8 and R2 cells, as well as parental SW480 cells, yielded colonies. R2 cells, however, yielded about ten times the number of large colonies (>0.25 mm in diameter) obtained with the E8 cells (Table 2).

Tumorigenicity of E- and R-Type Cells in Nude Mice and Histology of the Tumors. As shown in Table 3, the tumorigenicity of E8 cells was similar to that of the parental SW480 cell line. The R2 cells grew extremely rapidly in nude mice, so that by day 25 the tumors produced by the R2 cells were 6–9-fold larger than those produced by the SW480 or E8 cells.

Histological examination of tumors derived from the E8 and R2 cells showed that both tumors were basically poorly differentiated adenocarcinomas. However, in contrast to E8 tumors, the weights of the tumors that grew in nude mice at triplicate injection sites are shown in milligrams. Also shown are the mean values of the triplicates and the SDs for these triplicates.

Table 2 Growth in soft agar of E8 and R2 cells

<table>
<thead>
<tr>
<th>No. of colonies (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW480</td>
</tr>
<tr>
<td>Experiment 1</td>
</tr>
<tr>
<td>Experiment 2</td>
</tr>
</tbody>
</table>

a The number of colonies in 6.25 mm² areas of the plate, which were larger than 0.25 mm in diameter, were counted. The counting done in 5 randomly selected areas per plate, and the total number of colonies in 5 areas was determined. All assays were done on triplicate plates. The data indicate the mean values and SD for these triplicates.

b ND, not done. For additional details, see “Materials and Methods.”

c Statistically significant (P < 0.01).

Table 3 Tumorigenicity assays in nude mice

<table>
<thead>
<tr>
<th>Weight (mg) of the tumors (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW480</td>
</tr>
<tr>
<td>Experiment 1</td>
</tr>
<tr>
<td>Experiment 2</td>
</tr>
</tbody>
</table>

a The weights of the tumors that grew in nude mice at triplicate injection sites are shown in milligrams. Also shown are the mean values of the triplicates and the SDs. For additional details, see “Materials and Methods.”

b ND, not done.

c Statistically significant (P < 0.01).
Fig. 3. Histopathology of mouse tumors in nude mice that developed after subcutaneous injection of the SW480, E8, and R2 cell lines (H&E). The E8 tumor is shown at ×100 (A) or ×250 (C). This tumor displays a somewhat differentiated adenocarcinoma with well-formed glandular elements and well-preserved nuclear polarity. The R2 tumor is shown at ×100 (B) or ×250 (D). It displays a poorly differentiated adenocarcinoma. When compared to the E8 tumor, it shows no well-formed glandular structures, a loss of nuclear polarity, and a slightly higher nuclear:cytoplasmic ratio.

Fig. 4. Morpohologies of hybrid clones produced from the fusion of E8 and R2 cells. A–F, morphologies (×200) of six independently isolated E-R hybrid clones, clones ER-2, 3, 14, 17, 19, and 24, respectively. Their morphologies are quite similar to that of the R2 cells (Fig. 1D). Original magnification. ×200.
### Table 4  Cell fusion studies

<table>
<thead>
<tr>
<th>Cells used in the fusion</th>
<th>Colonies resistant to both G418 and hygromycin</th>
<th>Morphology of hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehygro × Rneo</td>
<td>100</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eneo × Ehygro</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Eneo × Eneo</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ehygro × Ehygro</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rneo × Rneo</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> The clonal lines E8 neo-10, E8 hygro-1, and R2 neo-3, which are resistant to G418 or hygromycin, were used in this study and are abbreviated Eneo, Ehygro and Rneo, respectively. For experimental details, see “Materials and Methods.”

<sup>b</sup> Five of these clones exhibited morphologies which were similar but not identical to that of the E-type cells; the morphology of one clone was exactly the same as that of the E-type cells.

which showed some glandular formation (Fig. 3, A and C), this differentiation marker was completely lacking in the R2 tumors (Fig. 3, B and D). In addition, the nucleuscyplytoplasmic ratio in the cells of the E8 tumors was smaller than that seen in the R2 tumors. These findings support the interpretation that the R2 tumors are less differentiated than E8 tumors.

**Cell Fusion Hybrids from E- and R-Type Cells.** To investigate possible genetic alteration(s) in R-type cells that account for their phenotype, we constructed cell hybrids between E8 and R2 cells. E-hygro and R-neo cells were fused, and hybrids were selected by growth in medium containing both G418 and hygromycin (see “Materials and Methods”). Ninety-four of the 100 hybrids obtained showed the same morphology as R2 cells, indicating that the R phenotype is dominant over the E phenotype. When 36 of the hybrid colonies with the R-type morphology were cloned and expanded, their rounded morphology persisted. Six of these hybrid clones, designated ER-2, 3, 14, 17, 19, and 24, were selected for further analysis. These E-R hybrids were similar to R2 cells not only in their morphology but also with respect to their growth rate and decreased adhesiveness. The morphology of these clones is shown in Fig. 4. Analysis of the six colonies (from the original 100 E-R hybrids; Table 4) that did not display the R-type phenotype revealed that five of these colonies had an intermediate phenotype and one had the E-type phenotype. However, upon clonal expansion in the double selection medium none of these clones survived, suggesting that they were not true hybrids. Thus, the frequency of the R-type phenotype in E-R hybrids may be even higher than 94%. Two kinds of control experiment were performed, in parallel with the cell fusions between E-hygro and R-neo cells. The first and most important control was fusion between E-neo and E-hygro cells. The resultant 24 E-E hybrid clones exhibited

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**Fig. 5.** Karyotypes of the E8 (A) and R2 (B) cell lines. Arrows, major rearranged chromosomes. For a detailed analysis, see Table 5.

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the typical E-type morphology, clearly indicating that the R-phenotype in E-R hybrids is not due to an artifact related to cell fusion or simply to a gene dosage effect. The second type of control was a series of fusions consisting of E-neo × E-neo, E-hygro × E-hygro, and R-neo × R-neo cells. All of these fusions failed to yield colonies resistant to both G418 and hygromycin, proving that the doses of these drugs used for selection were appropriate.

Karyotypic Analysis of SW480, E8, R2, and a E-R Hybrid. The modal chromosome number of the parental SW480 cells was 56, and the karyotype was similar, in general, to the karyotype of this cell line, published by Yaseen et al. (13). We did, however, see a few differences, and there are some differences in our interpretation of the same rearrangements. The karyotype of the E8 cells is shown in Fig. 5A. In E8 cells the modal number was 58, and the karyotype was very similar to that of the parental uncloned SW480 cell. Our interpretation of the karyotypic differences between the SW480 and the E8 cells is summarized in Table 5. A striking finding was that the chromosome counts in the R cell lines ranged from 94 to 99, and the karyotypes showed numerous changes from the E-type cells. Detailed analysis was performed on the subline R2 (Table 5; Fig. 5B). Certain shared abnormalities between the karyotypes of the E8 and R2 cell lines, and the fact that the R2 cells displayed duplications of several abnormal chromosomes present in the E cells, provided evidence that the R-type cells originally arose from E-type cells, presumably by polyploidization due to mitotic failure or cell fusion. On the other hand, certain features of the karyotypes do not support this interpretation. Thus, both cell lines contain only one chromosome 18, and single copies of several derived chromosomes are found in E8 cells. Furthermore, many of the rearrangements found in the E8 cells are not present in the R2 cells, while the R2 cells contain three analyzable rearrangements and nine marker chromosomes not present in the E8 cells.

The karyotype of the hybrid cell line ER24 was analyzed in detail (data not shown). The mean number of chromosomes per cell was about 130 (the range in the 5 cells analyzed was 129–145). The cells contained markers unique to both the E8 and R2 cell lines, thus proving that they were hybrids between the two cell lines. The presence or absence of several rearranged chromosomes in the ER24 cells is noted in Table 5. From this analysis we could exclude a relationship between the R phenotype and the abnormal chromosome 11 present in the R2 cells, since the hybrid line maintained the R phenotype but was lacking this chromosome. However, the existence of several marker chromosomes of unknown origin in both the R2 cell line and the hybrid made it impossible to establish a relationship between the R phenotype and other chromosomes or chromosomal rearrangements.

Flow Cytometric Analysis of E- and R-Type Cells and E-R Hybrids. Using flow cytometry, we determined the relative DNA content as calculated from the G1 peak fraction of E8 and R2 cells. The values were 59.6 and 95.8, respectively (Table 6). These values coincided very well with chromosome numbers, again showing that R2 cells were much more hyperploid than E8 cells. We performed a similar analysis on six independent E-R hybrid clones. The relative value of DNA content of these hybrids varied from 114.0 (ER-14) to 131.0 (ER-24). This is consistent with these clones being true hybrid lines. Considering the fact that the chromosome number and the relative DNA content of the ER-24 cells were very similar proportionately, we can reasonably assume that the relative DNA content of the other hybrids serves as an indicator of the approximate number of chromosomes these cells contain. Based on this assumption, we calculated the relative extent of DNA retention in the E-R hybrid clones by comparing their DNA contents to the sum of the DNA contents of E8 and R2 cells. As shown in Table 6, the E-R hybrid clones contained approximately 73.0–84.3% of the DNA contributed by the two parental cell types. These findings are consistent with previous evidence that hybrid cells do not necessarily contain all of the chromosomes derived from both parental cells (19).

Table 5 Summary of deviation from normal diploid karyotype in three cell lines

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>SW480</th>
<th>E8</th>
<th>R2 (modal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-1,+der(1)(1;9)(q25;q13)</td>
<td>Same as SW480</td>
<td>+1,+1</td>
</tr>
<tr>
<td>2</td>
<td>+der(2)(2;12)(q37;q13)</td>
<td>Same as SW480</td>
<td>+2,+2,+2der(2)(2;12)(q23;q13)</td>
</tr>
<tr>
<td>3</td>
<td>+del(3)(q11)</td>
<td>Same as SW480</td>
<td>+3 (often 4 copies of 3)</td>
</tr>
<tr>
<td>4</td>
<td>+7(4p)</td>
<td>Same as SW480</td>
<td>+4,+7(4p)</td>
</tr>
<tr>
<td>5</td>
<td>-5,+der(5)(5;20)(q15;p11)</td>
<td>Same as SW480</td>
<td>+6,+6</td>
</tr>
<tr>
<td>6</td>
<td>+ins(7)(7)(7;3)(q36)</td>
<td>Same as SW480</td>
<td>+7,+7,+7,+der(7)(7)(q36;7)</td>
</tr>
<tr>
<td>7</td>
<td>+der(7)(7;7)(q36)</td>
<td>Same as SW480</td>
<td>+8,+8</td>
</tr>
<tr>
<td>8</td>
<td>-8,+der(8)(8;7)(p11.2)</td>
<td>Same as SW480</td>
<td>+9</td>
</tr>
<tr>
<td>9</td>
<td>-9,+der(9)(1;9)(q25;q13)</td>
<td>Same as SW480</td>
<td>+11,+2der(11)(1;7;15;?)</td>
</tr>
<tr>
<td>10</td>
<td>-10</td>
<td>Same as SW480</td>
<td>-12,+12,+4inv(12)</td>
</tr>
<tr>
<td>11</td>
<td>+11</td>
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<td>+13,+13</td>
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<td>13</td>
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<tr>
<td>14</td>
<td>-14,+del(14)(q14)</td>
<td>Same as SW480</td>
<td>+15 (often 4 copies of 15)</td>
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<tr>
<td>15</td>
<td>+16</td>
<td>Same as SW480</td>
<td>+16,+16</td>
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<td>18</td>
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<td>Same as SW480</td>
<td>+2der(20)(5;20)(q15;p11)</td>
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<tr>
<td>19</td>
<td>+der(20)(5;20)(q15;p11)</td>
<td>Same as SW480</td>
<td>+2-4 copies of der(20)(5;20)(q15;p11)</td>
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<td>Same as SW480</td>
<td>+22 copies of 21</td>
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<td>21</td>
<td>+22</td>
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<tr>
<td>Marker</td>
<td>M1</td>
<td>Same as SW480</td>
<td></td>
</tr>
</tbody>
</table>

a Also present in the karyotype of the hybrid clone, ER24, which has the R phenotype.

b Not present in ER24.

c Many also present in ER24.
estimated as 110-130.

ods," stained with Giemsa, and counted.

of a variant subclone of the SW480 human colon cancer cell

DISCUSSION

This value was 155.4.

criteria the R-type cells are more malignant than the E-type

contaminant. This possibility is, however, unlikely because the

E- and R-type cells have been stable during numerous pass-

differentiation is dominant. This result is intriguing since thus far most of the

gene. Karyotypic analysis also

mutation (12), loss of the

Table 6 Number of chromosomes and DNA content of the E-R hybrid clones

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of chromosomes</th>
<th>Relative DNA content</th>
<th>Relative DNA retention in the hybrid clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>E8</td>
<td>58</td>
<td>59.6</td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>94-99</td>
<td>95.8</td>
<td></td>
</tr>
<tr>
<td>ER-2</td>
<td>NDd</td>
<td>128.4</td>
<td>82.6%</td>
</tr>
<tr>
<td>ER-3</td>
<td>ND</td>
<td>120.7</td>
<td>77.7%</td>
</tr>
<tr>
<td>ER-14</td>
<td>ND</td>
<td>114.0</td>
<td>73.9%</td>
</tr>
<tr>
<td>ER-17</td>
<td>ND</td>
<td>125.5</td>
<td>80.8%</td>
</tr>
<tr>
<td>ER-19</td>
<td>ND</td>
<td>126.5</td>
<td>81.4%</td>
</tr>
<tr>
<td>ER-24</td>
<td>130</td>
<td>131.0</td>
<td>84.3%</td>
</tr>
</tbody>
</table>

d Metaphase chromosomes were prepared as described in "Materials and Meth-
ods," stained with Giemsa, and counted.

This column indicates the relative values for DNA content calculated from the

× 100 and divided by the sum of the relative DNA contents of the E8 and R2 cells.

This value was 155.4.

ND, not determined precisely. Approximate number of chromosomes was

sated by their poor adhesiveness and increased detachment

t from the culture dish. According to Trainer et al. (21), who

examined 19 human colorectal cancer cell lines, colorectal cell

lines can be divided into three classes based on their malignant

properties. The SW480 cell line belongs to the second class

because it exhibits intermediate properties, but we should em-

phasize that this reflects the properties of the predominant

E-type cells. This classification is consistent with the fact that

the SW480 cell line was established from a Dukes' B colon

cancer (5). Previous studies indicate that this cell line displays

most of the genetic changes seen in advanced colon cancers,

including a K-ras mutation (10), c-mye amplification (11), a p53

mutation (12), loss of the DCC gene on chromosome 18 (22),

and loss of the APC gene on chromosome 5 (14). The R-type

cells might be particularly useful, therefore, for identifying ad-

ditional genetic changes involved in very late stages of tumor

progression.

It is well established that hybrid cells made by fusing tumor-

ogenic cells with nontumorigenic cells are frequently nontum-

origenic, suggesting the presence of tumor suppressor genes in

normal cells which are inactivated in tumorigenic cells (19).

We found, however, that when the R-type variant of SW480 cells

was fused with the E-type SW 480 cells the E-R hybrids ob-

tained had the R-type morphology. Thus, the R-type phenotype

is dominant. This result is intriguing since thus far most of the

generic changes seen in the later stages of tumor progression

appear to be recessive rather than dominant (3). We are hopeful

that further studies of the R-type variant of SW480 cells will

provide further insights into the process of tumor progression

as well as fundamental aspects of cell morphology, adhesion,

and growth control.

ACKNOWLEDGMENTS

We thank Thomas M. Delohery for valuable technical assistance in

performing the flow cytometric analysis and Antonio Sobrino for pre-

paring the karyotypes. We also express our appreciation to Drs. K-M.

Tchou-Wong and S. Doi for valuable comments and Sonia Tirado for

assistance in the preparation of the manuscript.

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