DNA Amplification and Tumorigenicity of the Human Melanoma Cell Line MeWo

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ABSTRACT

Homogeneously staining regions (HSRs) were found in hypodiploid cells (40%) of a subline of the human melanoma cell line, MeWo, (MeWo-C) but were absent from the hypotetraploid cells (60%). Another subline (MeWo-B) was also shown to contain two populations of cells, 70% hypodiploid and 30% hypotetraploid. None of the MeWo-B cells contained HSRs, but all four cell types from both sublines shared marker chromosomes indicating their common origin. The hypodiploid MeWo-B cells were karyotypically similar to the hypodiploid MeWo-C cells except for the presence of the HSRs in the latter. Both MeWo-C and MeWo-B sublines were injected into BALB/c nude mice. The MeWo-C cells were markedly more tumorigenic than MeWo-B cells as judged by tumor incidence, latency, average tumor size, and tumor take values. Cytogenetic and flow cytofluorometric analyses of the tumors induced by MeWo-C cells revealed a shift in the tumor cell population from 40% to >90% HSR-containing tumor take values. Cytogenetic and flow cytofluorometric analyses of the tumors induced by MeWo-B cells revealed a shift in the tumor cell population from 40% to >90% HSR-containing hypodiploid cells during tumor growth. Hybridization of tumor DNA to a probe (D15Z1), the sequence of which is amplified in the HSRs, also indicated an increase in the proportion of HSR-bearing cells during tumor growth. No such selective advantage was found with the hypodiploid, HSR-lacking MeWo-B cells. The results suggest that HSRs found in the human melanoma line MeWo may confer enhanced tumorigenicity to the cells containing them.

INTRODUCTION

HSRs and DMs are chromosomal abnormalities representing amplified DNA sequences. HSRs were first described in Chinese hamster ovary cells resistant to methotrexate (4), where the amplified DNA contained multiple copies of the dhfr gene (18). Other examples of amplification of genes conferring drug resistance include CAD in N-(phosphonacetyl)-L-aspartic acid-resistant cells (16), metallothionein in cadmium-resistant cells (2), and an M, 19,000 protein in vincristine-resistant cells (14). HSRs have also been found in solid tumors of rodent and human origin. HSRs in a mouse adrenocortical tumor and a human colon carcinoma have been shown to contain amplified c-Ki-ras (20) and c-myc (1) oncogenes, respectively. The greater majority of cell lines established from neuroblastomas have been shown to display either HSRs or DMs (3), and the N-myc oncogene was amplified up to 120-fold in 7 different neuroblastomas that were studied (15, 19).

It has been suggested that amplification of oncogenes in tumors might contribute to tumorigenesis and/or maintenance of the neoplastic phenotype (1, 15, 19, 20). The only direct study on the role of HSRs in tumor growth was conducted by Gilbert et al. (8) using a human retinoblastoma cell line. A progressive increase in the size of the HSRs was observed with continuing passage in nude mice. A positive correlation was also observed between the length of the HSRs and the tumorigenicity (latency) of the cells containing them.

Here, we present results of a study on the role in tumorigenicity of HSRs found in the human melanoma cell line, MeWo. The line was derived from a metastatic melanoma of a Caucasian male (7). A subline of MeWo (MeWo-C) cultured in our laboratory consisted of 2 populations of cells (22). The hypodiploid MeWo cells were HSR+ on the X chromosome and a der(15) chromosome, while the hypotetraploid population was HSR- (10, 22). The availability of a line that consisted of both HSR+ and HSR- cells provided an opportunity to test for selective growth of either type of cell during tumor growth. Another subline (MeWo-B) was also used, because it contained HSR- hypodiploid cells, which served as a further control. We present data which suggest that HSRs confer enhanced tumorigenicity to MeWo cells.

MATERIALS AND METHODS

Animals. BALB/c nude mice were bred in our laboratory in a pathogen-free barrier unit. Three-month-old females were used in all experiments.

Cell Lines and Culture Conditions. MeWo is a cell line derived from a lymph node metastasis in a patient with malignant melanoma (7). Cells have been obtained from Dr. J. Fogh at the Sloan-Kettering Institute for Research on Cancer. Two sublines have remained chromosomally stable since our first chromosomal analysis. MeWo-C cells were injected into BALB/c nude mice. The MeWo-B cells were HSR- on the X chromosome and a der(15) chromosome, while the hypotetraploid population was HSR- (10, 22). The availability of a line that consisted of both HSR+ and HSR- cells provided an opportunity to test for selective growth of either type of cell during tumor growth. Another subline (MeWo-B) was also used, because it contained HSR- hypodiploid cells, which served as a further control. We present data which suggest that HSRs confer enhanced tumorigenicity to MeWo cells.

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DNA Extractions from MeWo Cells and Placenta. DNA was extracted as described previously (22).

DNA Dot Blotting. Genomic DNA was sheared to produce random fragments 2 to 5 kb long with a Branson sonic power sonifier (Setting 5). D15Z1 DNA (22) was digested with Kpnl, and the sheared or cut DNA was diluted in 20-fold SSC. Genomic DNA (0.5 to 10 ng) and 0.01 to 0.15 ng of D15Z1 DNA was mixed with 1 μg of sheared herring sperm DNA in 20-fold SSC. The DNA mixture was denatured by boiling for 15 min and then quenched on ice. Nitrocellulose filters were wetted in distilled H2O, then in 2-fold SSC, and finally in 20-fold SSC. The filters were prewashed in 0.1% sodium dodecyl sulfate at 90° for 15 min to reduce nonspecific background. Wet nitrocellulose filters were placed in a BRL Hybridot dot-blot apparatus, and the DNA samples were applied to the filter under vacuum. D15Z1 was labeled with [α-32P]dCTP by the method of Rigby et al. (17) to specific activities of 1 to 6 × 10⁶ cpm/μg.

Flow Cytometry. Tumor cells or human peripheral blood lymphocytes were washed in phosphate-buffered saline and mixed with propidium iodide (50 μg/ml) in 0.1% sodium citrate at a concentration of 10⁶ cells/ml for 10 min at room temperature. Cells were filtered through nylon mesh, and 5 × 10⁶ to 10⁷ cells were analyzed on a Coulter EPICS-2 flow cytometer.

RESULTS

Chromosome Analyses. Cytogenetic analysis of MeWo-C revealed the presence of 2 cell types: 60% of the cells had approximately 83 chromosomes (hypotetraploid); and 40% had 43 chromosomes (hydroploid). With quinacrine fluorescence, all of the hydroploid, but none of the hypotetraploid, cells exhibited HSRs on an X chromosome and a derivative chromosome 15 (10, 22). Both cell types showed the same marker chromosomes (Fig. 1, A and B), including a translocation between chromosomes 12 and 15 (M1), a small pale-staining chromosome probably derived from a chromosome 22 (M2), and a third marker chromosome (M3), indicating their common origin. MeWo-B cells also contained these markers (Fig. 1, A and B). None of the MeWo-B cells contained HSRs; however, both types of hydroploid cells had a common marker 17 (M4), which differed from the marker 17's found in the hypotetraploid cells (M4a and M4b).

Growth of MeWo Cells in Nude Mice and in Vitro. The growth properties of MeWo-B and MeWo-C cells in BALB/c nude mice were compared. BALB/c nude mice (n = 32) were given injections s.c. with 8 × 10⁶ (2 mice/group), 10⁶, 5 × 10⁶, 2.5 × 10⁶, 1.25 × 10⁶, or 0.6 × 10⁶ (6 mice/group) cells/mouse, and tumors were measured every 3 to 10 days. As shown in Chart 1 and summarized in Table 1, MeWo-C was more tumorigenic than MeWo-B. The average tumor incidence was approximately twice as high, the average latency period was approximately 3 times shorter, the average tumor size was approximately 7 times greater, and the tumor take value was more than 15 times lower. Fig. 2 shows the typical appearance of MeWo-B and MeWo-C tumors 30 days after injection.

In order to exclude the possibility that MeWo-C cells simply divide more rapidly than MeWo-B cells, we compared the growth in vitro of MeWo-B and MeWo-C cells. The doubling time for both sublines was approximately 24 hr (Chart 2).

Dot Blot DNA Hybridization. During a previous study, we found that a member of the Kpnl family of repetitive DNA was amplified in the HSRs (22). D15Z1 (which contains a copy of the 1.8 kb Kpnl sequence) was used to screen DNAs for amplification of the 1.8 kb Kpnl sequence as an indicator for the presence of HSRs. DNA from MeWo-B, MeWo-C, and MeWo-B-induced tumors and MeWo-C-induced tumors and placenta (as a control) was hybridized with D15Z1. As shown in Fig. 3 and Table 2, the hybridization intensities of the MeWo-B and MeWo-B tumor DNAs were similar to that of placental DNA and represented approximately 6000 copies of D15Z1 per average diploid genome. There was a 2.5-fold increase in the number of 1.8-kb Kpnl sequences in MeWo-C cells and about a 10-fold increase in all 6 of the MeWo-C-induced tumors examined. These results suggest either growth of the HSRs and/or an increase in the number of HSR-containing cells in the MeWo-C-induced tumors.

Ploidy Analysis. Since only the hydroploid population of the MeWo-C cells contained HSRs, we measured the proportion of hydroploid cells in MeWo-C and MeWo-C-induced tumors by flow cytometry. As shown in Chart 3, the proportion of hydroploid cells shifted from 40% in the MeWo-C cultures to 90% in the MeWo-C-induced tumors. The results were the same for all 14 MeWo-C-induced tumors examined. The cytogenetic analysis showed that the HSR⁺ hydroploid cells represented 98% of the MeWo-C tumor cells (Table 2). The ploidies of the MeWo-B cells and MeWo-B-induced tumor cells (6 individual
Table 1
Comparative tumorigenicity of MeWo-B and MeWo-C cells

<table>
<thead>
<tr>
<th>No. of cells injected</th>
<th>Tumor incidence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Latency&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Tumor size&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Tumor take&lt;sup&gt;d&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MeWo-B&lt;sup&gt;e&lt;/sup&gt;</td>
<td>MeWo-C&lt;sup&gt;f&lt;/sup&gt;</td>
<td>MeWo-B</td>
<td>MeWo-C</td>
</tr>
<tr>
<td>8 x 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>2/2</td>
<td>2/2</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>1 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>4/6</td>
<td>6/6</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>5 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3/6</td>
<td>6/6</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>2.5 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3/6</td>
<td>6/6</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>1.25 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3/6</td>
<td>6/6</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>0.6 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3/6</td>
<td>5/6</td>
<td>30</td>
<td>14</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of mice receiving injections that developed tumors (30 days after injection).

<sup>b</sup> Time, in days, to development of 1-cm³ tumor volume.

<sup>c</sup> Average (per concentration group) tumor size in cm³ 30 days after injection.

<sup>d</sup> Number of tumor cells required to induce tumors in 75% of animals.

<sup>e</sup> MeWo-B-induced tumors.

<sup>f</sup> MeWo-C-induced tumors.

<sup>g</sup> Mean ± S.E.

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tumors) were also measured by flow cytofluorometry. The proportion of hypodiploid cells shifted from 70% in the MeWo-B subline to 0% in the MeWo-B-induced tumor cells (Chart 3).

**DISCUSSION**

This study was designed to examine the effect of the HSRs found in the human melanoma cell line MeWo on its tumorigenic potential. For this purpose, 2 different sublines were used. The MeWo-B subline contained 70% hypodiploid, HSR<sup>+</sup> cells and 30% hypotetraploid, HSR<sup>-</sup> cells. The MeWo-C subline had 40% hypodiploid, HSR<sup>+</sup> cells and 60% hypotetraploid, HSR<sup>-</sup> cells. Such chromosomal heterogeneity of the MeWo sublines is a common feature of many malignant tumors and the cell lines derived from them. The pattern of the chromosomal markers in the 2 sublines demonstrated a common origin of all 4 cell types. Growth of MeWo-C cells in nude mice resulted in the selection of the HSR<sup>+</sup> hypodiploid cells over the HSR<sup>-</sup> hypotetraploid cells as detected by flow cytofluorometry, cytogenetic analysis, and hybridization of tumor DNA with a cloned KpnI family member which comprises part of the MeWo HSRs (22). In contrast, the tumors induced by MeWo-B cells consisted only of hypotetraploid cells. These results suggest that the presence of the HSRs in the hypodiploid population of MeWo-C confers upon these cells properties allowing their selection over hypotetraploid cells. This suggestion is consistent with the observation that the cells containing HSRs grew in nude mice at a faster rate, with a shorter latency, higher tumor incidence, and lower tumor take than the MeWo cells lacking HSRs. It is probable that the HSRs found in this cell line play a role in tumor progression, rather than tumor initiation, since HSR<sup>-</sup> MeWo cells are also tumorigenic. However, we cannot rule out the possibility that another difference may be responsible for the enhanced tumorigenicity of HSR<sup>+</sup> MeWo cells.

These results may be added to the growing body of data supporting the hypothesis that amplification of genetic material...
DNA AMPLIFICATION AND TUMORIGENICITY

Table 2

<table>
<thead>
<tr>
<th></th>
<th>% of hypotetraploid cells</th>
<th>% of hypodiploid cells</th>
<th>% of HSR</th>
<th>Copy no. of D15Z1 (x 10^3)</th>
<th>Relative copy no. of D15Z1</th>
</tr>
</thead>
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<tr>
<td>Placenta</td>
<td>30</td>
<td>70</td>
<td>0</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>MeWo-B</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>1.0</td>
</tr>
<tr>
<td>MeWo-B tumors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MeWo-C</td>
<td>60</td>
<td>40</td>
<td>38</td>
<td>15</td>
<td>2.5</td>
</tr>
<tr>
<td>MeWo-C tumors</td>
<td>10</td>
<td>90</td>
<td>98</td>
<td>70</td>
<td>11.5</td>
</tr>
</tbody>
</table>

* Cells (5 x 10^6 to 10^7) analyzed by flow cytofluorometry.
* Cells (100 to 250) of each sample were used for cytogenetic analysis.
* The numbers are given per average (diploid) genome. Measured using a standard dot-blot hybridization curve with D15Z1.
* Representative results for 6 MeWo-B and 14 MeWo-C independently derived tumors.

Placenta

MeWo-C

MeWo-C tumor

MeWo-B

MeWo-B tumor

Fig. 3. Dot-blot hybridization of MeWo DNA with D15Z1. Placental, MeWo-C, MeWo-C tumor, MeWo-B, and MeWo-B tumor DNAs were hybridized to [α-32P]dCTP-labeled D15Z1. Genomic DNA diluted in 20-fold SSC was sheared to produce random fragments 2 to 5 kb long. Sheared DNA (0.5 to 10 ng) was mixed with 1 ug (200 µl) of sheared herring sperm DNA. The mixtures were boiled for approximately 15 min, applied to nitrocellulose filters, and probed with nick-translated D15Z1.

may contribute to the expression of the neoplastic phenotype. Recently, there have been many demonstrations of amplified genetic material in a variety of primary tumors and tumor-derived cell lines. Cells from many cancers, including human mammary, esophageal, pharyngeal (12), stomach, colon, cervix, and vulva cancers (24), neuroblastomas (11, 19), lung carcinomas (13), and leukemias (6), exhibit amplification of genetic material in the form of either HSRs or DMs. In addition, the presence of an HSR on chromosome 1 (9) and elongation of an HSR on chromosome 3 (8) in human retinoblastoma lines are directly correlated with an increase in tumorigenicity.

Some HSRs and DMs have been shown to contain amplified oncogenes. N-myc amplification has been detected in several neuroblastoma lines and primary tumors from patients who have not received chemotherapy (11, 15). Extra copies of the myc gene were found in a human myeloid leukemia line (6) and a more malignant type of small cell lung carcinoma (13). Amplification has also been demonstrated in the neuroendocrine cells of a human colonic carcinoma (1), and the ras gene was amplified in a mouse adrenal cortex tumor line (20) and in a human bladder cancer line.4 Amplified abl gene was found in a chronic myelogenous leukemia cell line (21). We are presently examining the HSR MeWo cells for amplification of known oncogene sequences.

Amplification of genetic material varies with respect to the degree of amplification, the sequence amplified, and the chromosomal location of the amplified region even in primary tumors and cell lines originating from the same tissues. In 2 reported cases of HSRs in primary melanomas, an HSR was located on the long arm of chromosome 2 (5) and the other on chromosome 7 (23). Some qualitative and quantitative differences in amplification were shown to exist among several neuroblastoma lines (15), and although some tumors share common amplified sequences (e.g., N-myc and myc in neuroblastomas and small cell lung carcinoma, respectively), many HSR-bearing tumor cells

4 J. Mak, personal communication.
have unrelated sequences amplified (15) and do not exhibit increased copy number of any known oncogenes.

The numerous examples of DNA amplification in primary tumors and tumor lines suggest a role for structures like HSRs in the maintenance and/or enhancement of the malignant phenotype. Such effects are probably gene-product mediated. Amplification of different DNA sequences in different cancer cells implies that a variety of genes may be involved. Therefore, a detailed study of HSRs in each separate case might yield important information on the mechanisms by which amplified DNA affects tumor development.

The HSRs studied in this work were probably present in the original melanoma tumor from which the MeWo line was derived, since the earliest available passage, passage 14, of MeWo contains a population of cells with an HSR on chromosome 15.5

The demonstration that HSR-containing cells are strongly selected during growth of melanoma tumors in nude mice and that these cells exhibit enhanced tumorigenicity over HSR− MeWo cells, further suggests that certain genes amplified in the HSRs play an important role in augmented expression of the neoplastic phenotype of the HSR-positive cells. About 80% of the DNA comprising the HSRs has been characterized (10, 22).

It has been shown that the amplified unit consists of the short arm and the centromere of chromosome 15 (10). The identified sequences include large blocks of a tandemly repeated 1.8-kb KpnI family member (centromeric heterochromatin) and nucleolar organizer regions, consisting of rRNA genes. Currently, we are trying to identify and characterize genes, amplification of which may result in the enhanced tumorigenicity of the melanoma line MeWo.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Metaphase cells stained with quinacrine-HCl illustrating the chromosome abnormalities found in MeWo-B and MeWo-C sublines. Some common marker chromosomes are indicated. M1 represents a translocation between a chromosome 15 and the long arm of a chromosome 12. M2 is a small, pale staining chromosome, probably derived from chromosome 22 as no normal 22s are found in the MeWo cells. M3 is another common marker of unknown chromosomal origin. M4 is composed of a chromosome 17 with an additional band at the distal end of the long arm. M4a has a further duplication of this band, and M4b appears to have arisen by translocation of M4 with the long arm of chromosome 10. A, MeWo-B hypodiploid cell; B, MeWo-B hypotetraploid cell; C, MeWo-C hypodiploid cell; and D, MeWo-C hypotetraploid cell.
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