Effect of Nucleolar P120 Expression Level on the Proliferation Capacity of Breast Cancer Cells

Anna Fonagy, Carol Swiderski, Alexander M. Ostrovsky, Wade E. Bolton, and James W. Freeman

Department of Surgery, Division of General Surgery, Lucille Parkey Markey Cancer Center, 40536 [A. F., C. S., A. M. O., J. W. F.,] and Coultier Immunology, Hialeah, Florida 33010 [W. B.]

ABSTRACT

Steady-state level of nucleolar P120 protein and P120 mRNA was compared to the doubling time and S-phase fraction in human breast cancer cell lines growing exponentially and in similar cells treated with a single dose of P120 antisense oligodeoxynucleotides. The study included six breast cancer cell lines and one nontransformed breast cell line with doubling times from 1.1 to 5.5 days and with S-phase fractions from 35 to 9%. P120 expression level was determined by densitometric computerized evaluation of protein and mRNA blots and with a quantitative 32P-reverse transcriptase-polymerase chain reaction method developed for small-scale samples. In the slowest growing normal cell line, P120 expression level was only about 10% of the level found in the most rapidly growing cancer cell line. The amount of P120 mRNA was highly correlated with the amount of P120 protein (P = 0.0001), indicating that P120 accumulation is regulated in these cells primarily at a transcriptional level. There was also a significant positive correlation between the level of P120 protein/mRNA and doubling time of cell lines (P = 0.0008) or percentage of S-phase cells (P = 0.0210). P120 antisense oligomer treatment decreased the growth rate of cells in a dose-dependent manner, and the inhibition reached 70% at 100 μM concentration. Both P120 mRNA and P120 protein levels were also decreased by approximately 70% in cells treated with 100 μM P120 antisense oligomer. Slowly growing cells exhibited 50% inhibition by treatment at a proportionally lower concentration of P120 antisense oligomer than fast growing cells. This study shows that the expression of P120, measured either at the protein or the mRNA level, correlates with proliferation rate, identifying P120 as a cell proliferation marker.

INTRODUCTION

Proliferation-associated nucleolar protein P120 was identified after development of a monoclonal antibody to human tumor nucleoli (1). This antibody recognized a novel M, 120,000 nucleolar protein that was detectable in a broad range of tumors and proliferating cells but not in most normal, resting cells or tissues (1). cDNAs and genomic clones for P120 have been isolated and characterized (2, 3). The properties of P120 have been recently reviewed by Busch (4) and Freeman and Busch (5).

P120 sense and antisense constructs (6–8), as well as P120 antisense oligonucleotides, have been generated. Transfection with a P120 antisense construct caused a decreased growth rate in vitro in NIH/3T3 cells and decreased tumor growth in vivo (8). In a synchronized cell population, a peak amount of P120 protein was found in S-phase cells by flow cytometric and Western blot analysis (9, 10). Antisense-mediated specific inhibition of P120 expression caused a block of cell cycle progression at the G1/S border in mitogen-stimulated lymphocytes (11). Although the specific biological role of P120 is not known, some evidence suggests that P120 is essential in the ribosomal biogenesis (12). These studies suggest that P120 is necessary for cell cycle progression and may serve as a proliferation marker.

The level of P120 in breast cancer may relate to the proliferative properties of the tumor. The S-phase fraction has been reported to be an important prognostic marker in breast cancer (13, 14). The high level of P120 expression in S-phase cells (9, 10) suggests that the level of P120 expression may serve as a biological marker for cell proliferation. In a retrospective breast cancer study, P120 expression determined by immunohistochemistry was found to be associated with patient prognosis (15). Node-positive patients with high P120 expression had the worst survival rate, and node-negative patients with tumors that showed a low level of P120 expression had the best survival (94%, 5-year survival). Node-negative patients whose tumors were immunopositive for P120 had a poor survival rate (67%, 5-year survival). Therefore, node-negative patients whose tumors are negative for P120 may not need adjuvant therapy.

The purpose of this study was to determine whether P120 was directly correlated with proliferation properties. Proliferation rate and P120 expression, at both the protein and the mRNA levels, were systematically and quantitatively examined in seven breast cancer cell lines with different growth rates. A significant correlation was found between the steady-state levels of P120 mRNA and P120 protein and between P120 expression and proliferation rate. The potential biological and clinical implications of these findings are discussed.

MATERIALS AND METHODS

Cell Lines. BT 549, HBL 100, MCF-7/6, MCF-7/AZ, Hs 578T, DU 4475, and Hs 578N cell lines were purchased from American Type Culture Collection. Medium content, seeding density, fluid renewal, etc. were determined according to the instructions of the product sheets.

Probes and Amplifiers. A 1.8-kilobase human P120 cDNA EcoRI fragment and a 0.9-kilobase chicken ß-actin cDNA HindIII-EcoRI fragment were used in Northern and slot blots. The P120 primer pair was chosen by the “Oligo” computer program and made at Macromolecular Synthesis Laboratory, University of Kentucky Medical Center (Lexington, KY.). The sense primer was from 1734 to 1753 nucleotides, and the antisense primer was from 2316 to 2333 nucleotides of P120 cDNA generating a 600-base pair DNA fragment. The human GAPDH primer pair was purchased from Stratagene (La Jolla, CA).

Oligomers. The antisense oligomer used was a pentadecadeoxyribonucleotide (5'-AAAGCCCCCCCCAC-3') complementary to nucleotides of P120 cDNA immediately after the 11th splice junction site (2, 3, 11). The nonsense oligomer was a random mixture of the same nucleotides (5'-ACGCCCCAC-3'; Macromolecular Synthesis Laboratory). MTT Assay. Media were removed from wells. MTT in media was added at a 0.5-μg/ml final concentration (16, 17). After 2 h incubation at 37°C, excess MTT was removed, and washed cells were subjected to lysis in dimethyl sulfoxide. Color intensity was measured on an enzyme-linked immunosorbent assay reader at 590 nm.

PAGE and Western Blot. Washed cells were resuspended in Laemmli electrophoresis solubilization buffer (18) at a concentration of 2 x 106 cells/ml and were then incubated at 100°C for 3 min. Cell lysates were subjected to electrophoresis in 7.5% polyacrylamide gels containing 0.1% sodium dodecyl sulfate as described by Takacs (19). Western blotting was performed by using modifications (1, 10, 11) of the method of Towbin et al. (20). Equal loading

Received 10/22/93; accepted 2/3/94.

The cost of publication of the article was defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by a grant CA-49633 from the USPHS—National Cancer Institute.
2 To whom correspondence should be addressed, at Combs Research Facility, Room 313, University of Kentucky Medical Center, 800 Rose Street, Lexington, KY 40536-0093.
3 The abbreviations used are: cDNA, complementary DNA; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse transcriptase-polymerase chain reaction.
was confirmed by silver staining (21) of the separated proteins from the same samples on separate gels.

Flow Cytometry. One million cells/tube were washed with phosphate-buffered isotonic NaCl solution (pH 7.4) and then resuspended in Couter DNA-Prep stain (Coulter Immunology, Hialeah, FL) containing propidium iodide. Cells were analyzed using an upgraded Profile 1 flow cytometer fitted with an argon ion laser and adjusted to emit 15 mW at 488 nm. Compartmentalization of the cell cycle was determined using an Epics Elite workstation with the "Multicycle" software developed by Peter S. Rabinovich (Phoenix Flow Systems, San Diego, CA).

Northern Blot and Slot Blot Analysis. Total RNA was prepared from cells by the method of Chirgwin et al. (22) by a guanidinium isothiocyanate lysis and subsequent CsCl gradient ultracentrifugation (Sorvall RC 70). After extraction and precipitation, the amount of RNA was determined spectrophotometrically at 260 nm and loaded equally. RNA was fractionated through 2.2 m formaldehyde-1% agarose gels and blotted onto nitrocellulose membranes or blotted directly in a slot blot apparatus (Schleicher & Schuell, Keene, NH). Filters were baked, prehybridized, hybridized in buffer containing 50% formamide, and washed according to standard procedures (23).

Radioactive probes were prepared by the random priming method (24, 25) with a kit from Boehringer Mannheim (Indianapolis, IN) using [alpha-32P]dCTP, tetra-triethyl-ammonium salt (specific radioactivity, 3000 Ci/mmol; ICN Biomedical, Inc., Costa Mesa, CA). Autoradiography was done on Kodak XAR-5 film at -70°C with intensifying screens. After each hybridization with P120 probe and autoradiography, RNA blots were reprobed for equal loading with a beta-actin DNA fragment.

Densitometry. Western, Northern, and slot blots were scanned directly by a Bio Image, Visage 2000 (Millipore Corp.) analytical instrument. Whole-band computer analysis was performed and the intensity of the bands determined.

cDNA Synthesis. Total cellular RNA (6 μg) was transcribed with reverse transcriptase in the presence of the 4 deoxynucleotide triphosphates, RNase inhibitor, and 6 mM MgCl2. Random hexamers were used as primers for the reaction at room temperature for 10 min, at 42°C for 15 min, and then at 99°C for 5 min. The reactions were stopped by cooling to 5°C and used in amplification according to the instructions accompanying the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT).

Quantitative RT-PCR. Aliquots of cDNA mixtures equivalent to 1 μg of starting RNA were divided into 100 μL of PCR reaction mixtures containing 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus), the corresponding primer pair in 1 μM concentration, and 10 μCi of [alpha-32P]dCTP (specific radioactivity, 3000 Ci/mmol; ICN Biomedical) in addition to the four previously added dNTPs. Amplifications were carried out with 15, 20, and 25 cycles of 45 s at 94°C, 45 s at 60°C, and 1.5 min at 72°C with a final extension of 10 min at 72°C. The PCR products were analyzed by a nondenaturing 8% PAGE. After autoradiography, individual bands of P120 and the constitutively expressed GAPDH were excised and quantified by liquid scintillation counting. The radioactivity incorporated was plotted against cycle number and extrapolated to zero cycle. Finally, the ratio of P120 to the control was determined.

RESULTS

Proliferation Rate of Breast Cell Lines. The growth rates of seven breast cell lines were determined by cell numbers (Fig. 1). For each cell line, different numbers of cells were plated into identical wells of a 24-well plate in numbers as stated in the legend of Fig. 1. Cells were then counted from separate wells every day for 6 days. DU 4475 was the only cell line that grew in cell suspension as floating curves and arc shown in Table 1. The doubling time for the fastest growing cell line, BT 549, was 1.1 ± 0.2 (mean ± SD) day and that for the slowest growing cell line, Hs 578N, was 5.5 ± 1.1 day. The other cell lines grew with a descending growth rate between the two in an order of HBL 100, MCF-7/6, MCF-7/AZ, Hs 578T, DU 4475, and 30,000 for Hs 578N. Cell numbers of each cell line were normalized to 40,000 cells plated. Duplicate wells were used for each point. Cells were harvested, stained with trypan blue, and counted every 24 h. Points (bars), means (±SD) of three separate experiments.

Table 1. Doubling times of exponentially growing cells in breast cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Doubling time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT 549</td>
<td>1.10 ± 0.20</td>
</tr>
<tr>
<td>HBL 100</td>
<td>1.15 ± 0.20</td>
</tr>
<tr>
<td>MCF-7/6</td>
<td>1.35 ± 0.25</td>
</tr>
<tr>
<td>MCF-7/AZ</td>
<td>1.40 ± 0.30</td>
</tr>
<tr>
<td>Hs 578T</td>
<td>1.70 ± 0.35</td>
</tr>
<tr>
<td>DU 4475</td>
<td>1.90 ± 0.40</td>
</tr>
<tr>
<td>Hs 578N</td>
<td>5.50 ± 1.10</td>
</tr>
</tbody>
</table>

* Doubling times were calculated in days from the growth curves of breast cell lines shown in Fig. 1. Results are means ± SD.
were estimated by computer analysis. Data represent percentages of total cells analyzed; results are means ± SD for sample data from a minimum of three separate experiments.

Cells were plated as described in the legend of Fig. 1. Tumor cells were harvested on the fourth day, and normal cells were harvested on the sixth day. One million cells from each cell line were stained with propidium iodide. DNA histograms were generated from fluorescent-activated cell sorter analysis of cells. The fractions of cells in G1-, S-, and G2/M-phases were estimated by computer analysis. Data represent percentages of total cells analyzed; results are means ± SD for sample data from a minimum of three separate experiments.

Table 2. Cell phase distribution of exponentially growing cells in breast cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>G1 (%)</th>
<th>HBL 100</th>
<th>MCF-7/6</th>
<th>MCF-7/AZ</th>
<th>HS 578T</th>
<th>DU 4475</th>
<th>HS 578N</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT 549</td>
<td>46 ± 9</td>
<td>56 ± 11</td>
<td>65 ± 13</td>
<td>66 ± 13</td>
<td>69 ± 14</td>
<td>60 ± 12</td>
<td>85 ± 17</td>
</tr>
<tr>
<td>HBL 100</td>
<td>35 ± 7</td>
<td>27 ± 5</td>
<td>24 ± 5</td>
<td>20 ± 4</td>
<td>18 ± 4</td>
<td>25 ± 5</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>MCF-7/6</td>
<td>19 ± 4</td>
<td>17 ± 3</td>
<td>11 ± 2</td>
<td>14 ± 3</td>
<td>13 ± 3</td>
<td>15 ± 3</td>
<td>6 ± 1</td>
</tr>
</tbody>
</table>

Table 3. Amount of P120 protein in exponentially growing cells of breast cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Absorbance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT 549</td>
<td>1.65 ± 0.35</td>
</tr>
<tr>
<td>HBL 100</td>
<td>1.45 ± 0.30</td>
</tr>
<tr>
<td>MCF-7/6</td>
<td>1.30 ± 0.25</td>
</tr>
<tr>
<td>MCF-7/AZ</td>
<td>1.00 ± 0.20</td>
</tr>
<tr>
<td>HS 578T</td>
<td>0.80 ± 0.15</td>
</tr>
<tr>
<td>DU 4475</td>
<td>0.50 ± 0.10</td>
</tr>
<tr>
<td>HS 578N</td>
<td>0.20 ± 0.04</td>
</tr>
</tbody>
</table>

* Western blots, as one representative shown in Fig. 2, were densitometrically analyzed. Data are given as means ± SD of a minimum of three separate experiments.

Fig. 2. Protein patterns and P120 protein levels in exponentially growing cells of breast cell lines. A, silver-stained PAGE; B, Western blot. Lane 1, BT 549; lane 2, HBL 100; lane 3, MCF-7/6; lane 4, MCF-7/AZ; lane 5, HS 578T; lane 6, HS 578N; lane 7, DU 4475. Cells were plated as described in the legend of Fig. 1. Cells were harvested on the third or fourth day for tumor cells and on the sixth day for normal cells. Cells were counted, subjected to lysis, and loaded into wells of polyacrylamide gel. Proteins were separated in duplicate gels: 75,000 cells were loaded for silver staining and 200,000 cells for Western blot. After electrophoresis, gels were processed as described in "Materials and Methods."

Fig. 3. Steady-state levels of mRNA in exponentially growing cells of breast cell lines. A, Northern blot; B, slot blot; C, 32P-RT-PCR. Lane 1, BT 549; lane 2, HBL 100; lane 3, MCF-7/6; lane 4, MCF-7/AZ; lane 5, HS 578T; lane 6, HS 578N; lane 7, DU 4475. β-Actin was used as control for Northern and slot blot and GAPDH for 32P-RT-PCR. Cells were plated and harvested as described in the legend of Fig. 1, except 175-cm² flasks were used, and the number of cells plated was proportionally enlarged. Immediately after cell collection, total RNA was prepared or cells were frozen in liquid nitrogen. Forty µg of total RNA was used for Northern and slot blot and 1 µg for cDNA synthesis in 32P-RT-PCR. Details for RNA preparation, electrophoresis, blotting, hybridization, probes, cDNA synthesis, amplification, amplifiers, and quantitative evaluations are given in "Materials and Methods."

Table 4. The BT 549 cell line had the highest value for each measured parameter; therefore, it was chosen as a 100% reference point. The values for the other cell lines were given as a percentage of the value of BT 549. According to the calculated Pearson correlation coefficient, a highly significant positive correlation was found between the steady-state level of P120 protein and P120 mRNA (P = 0.0001) in the seven cell lines. Similarly, a very strong positive correlation was observed for the percentage of S-phase cells and P120 protein or P120 mRNA content, but the correlation coefficients were smaller (P = 0.0210 and 0.0164, respectively) than those of P120 protein and P120 mRNA or doubling time (P = 0.0001).

Inhibition of Growth, P120 Protein, and P120 mRNA Content by P120 Antisense Oligodeoxynucleotide Treatment. Exponentially growing cells were treated with either P120 antisense or nonsense oligomer. Control cells were kept in a similar manner without treatment. Nonsense-treated cells were always compared with control cells. The measured parameters showed only a few percentage points of difference between nonsense oligomer-treated and control cells.
showed an approximately 70% decrease in P120 protein in cells extended treatment with a lower concentration of P120 antisense (50 μM) resulted in a decrease in proliferation rate without causing a corresponding decrease in cell proliferation. These data support the hypothesis that P120 is not only correlated with growth but is also involved in the proliferative capacity of human breast cancer cells. A role for P120 in cell proliferation is also supported by two previous studies (8, 11). First, fibroblasts transfected with the P120 sense gene constructs showed an increase in growth rate, and a subsequent transfection of these same cells with P120 antisense constructs resulted in a decrease of cell growth rate (8). Second, a study using mitogen-stimulated lymphocytes showed that antisense-mediated specific inhibition of P120 expression prevented the transition from G1 to S-phase (11). Thus, these studies collectively support the premise that P120 plays a role in the proliferation capacity of cells.

The inhibition of growth rate by P120 antisense oligomer was measured in three cell lines: one with a high growth rate (HBL 100), another with a medium growth rate (MCF-7/AZ), and a third with a low growth rate (Hs 578N). Exponentially growing cells were treated with a single dose of oligomers, 2 days after plating at a density, as stated in the legend of Fig. 4. Oligomer concentrations were maintained at a final 25, 50, 75, and 100 μM. Two days after treatment, cell viability was determined by trypan blue exclusion and an MTT assay was carried out. Inhibition was calculated as the percentage of antisense-treated values compared to the nonsense-treated values. Three separate experiments were carried out, and SD was calculated.

The inhibition of growth rate by P120 antisense oligomer was measured in three cell lines: one with a high growth rate (HBL 100), another with a medium growth rate (MCF-7/AZ), and a third with a low growth rate (Hs 578N). Exponentially growing cells were treated with a single dose of oligomers, 2 days after plating at a density, as stated in the legend of Fig. 4. Oligomer concentrations were maintained at a final 25, 50, 75, and 100 μM. Two days after treatment, cell viability was determined by trypan blue exclusion and an MTT assay was carried out. Inhibition was calculated as the percentage of antisense-treated values compared to the nonsense-treated values. Three separate experiments were carried out, and SD was calculated.

Table 4 Amount of P120 mRNA in exponentially growing cells of breast cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Northern blotsa</th>
<th>Slot blotsb</th>
<th>Radioactivity in 32P-RT-PCRc</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT 549</td>
<td>1.85 ± 0.35</td>
<td>1.30 ± 0.25</td>
<td>0.53 ± 0.10</td>
</tr>
<tr>
<td>HBL 100</td>
<td>1.75 ± 0.35</td>
<td>1.10 ± 0.20</td>
<td>0.49 ± 0.10</td>
</tr>
<tr>
<td>MCF-7/6</td>
<td>1.50 ± 0.30</td>
<td>0.90 ± 0.20</td>
<td>0.38 ± 0.10</td>
</tr>
<tr>
<td>MCF-7/AZ</td>
<td>1.10 ± 0.20</td>
<td>0.90 ± 0.20</td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>Hs 578T</td>
<td>1.20 ± 0.25</td>
<td>0.60 ± 0.10</td>
<td>0.27 ± 0.05</td>
</tr>
<tr>
<td>DU 4475</td>
<td>0.60 ± 0.10</td>
<td>0.40 ± 0.08</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>Hs 578N</td>
<td>0.30 ± 0.05</td>
<td>0.10 ± 0.02</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

* Northern blots, as one representative shown in Fig. 3A, were densitometrically analyzed. Data are given as means ± SD of a minimum of three separate experiments. The difference in the amount of actins is smaller than SD.

DISCUSSION

This study showed that the level of P120 expression correlated directly with the proliferation capacity of seven separate breast cell lines. Furthermore, decreasing the level of P120 expression by treatment with P120 antisense oligodeoxyribonucleotide produced a corresponding decrease in cell proliferation. These data support the hypothesis that P120 is not only correlated with growth but is also involved in the proliferative capacity of human breast cancer cells. A role for P120 in cell proliferation is also supported by two previous studies (8, 11). First, fibroblasts transfected with the P120 sense gene constructs showed an increase in growth rate, and a subsequent transfection of these same cells with P120 antisense constructs resulted in a decrease of cell growth rate (8). Second, a study using mitogen-stimulated lymphocytes showed that antisense-mediated specific inhibition of P120 expression prevented the transition from G1 to S-phase (11). Thus, these studies collectively support the premise that P120 plays a role in the proliferation capacity of cells.

In the present study, seven separate breast cell lines were used to correlate the endogenous level of P120 expression with proliferation properties. One cell line was a nontransformed, normal breast cell line with a very slow growth rate. The remaining six breast cancer cell lines, on the basis of their growth properties, could be placed into three subgroups: cell lines with a rapid growth rate (BT 549, HBL 100), an intermediate growth rate (MCF-7/6, MCF-7/AZ), and a slow growth rate (Hs 578T, DU 4475). A direct relation between the percentage of S-phase cells and growth rate was observed for six of...
the seven cell lines. Those cells that showed a rapid growth rate also exhibited a high percentage of S-phase cells. The one exception was DU 4475, which contained a higher percentage of S-phase cells than expected on the basis of doubling time. This could be explained if the S-phase of DU 4475 cells was longer than other cell cycle phases.

The level of P120 protein measured from Western blots and the level of P120 mRNA calculated from Northern blots and mRNA slot blots were highly correlated with growth rate and percentage of S-phase cells. 32P-RT-PCR quantitative measurements for P120 mRNA level were in agreement with Northern blot and slot blot analyses. The purpose of this alternative method for measuring P120 mRNA levels was to be able to assess P120 mRNA levels when the number of cells available for RNA preparation was small. The very sensitive RT-PCR method is especially useful for antisense-treated cells that require expensive oligonucleotides and also for analyzing small tissue samples or needle biopsies. Furthermore, because P120 mRNA and P120 protein levels were found to be highly correlated, the RT-PCR method, which measures P120 mRNA content, could be more accurate and precise than Western blot analysis or immunohistochemistry, which evaluates P120 protein levels, for assessing proliferation properties in the early phase of breast cancer.

One might expect that decreasing P120 expression by P120 antisense treatment would decrease the proliferative capacity of breast cells, if P120 endogenous levels are directly associated with growth rate and if P120 plays a necessary role in cell proliferation. To explore this possibility, three breast cell lines [one fast growing (HBL 100), one with an intermediate growth rate (MCF-7/AZ), and one slow growing (Hs 578N)] were treated with various concentrations (25–100 μM) of P120 antisense oligodeoxynucleotide. Decreasing the P120 level by a 2-day antisense treatment caused a decrease in proliferation rate but not in cell viability. An extended treatment (>3 days) of cells with 100 μM P120 antisense oligomer resulted in not only decreased cell proliferation rate but also decreased cell viability. This decrease in cell viability was not observed in nonsense-treated cells. As might be expected, the cell line HBL 100, which expressed a high level of P120, required almost twice as much antisense oligonucleotide to exhibit the same percentage of inhibition of proliferation rate as the Hs 578N cell line, which had only a low level of endogenous P120. In conclusion, the amount of P120 appears to influence the proliferation rate and a critical level of P120 appears to be necessary for continued cell viability.

Numerous studies suggest that the proliferation capacity of breast cancer cells, especially measured as a percentage of S-phase, is of prognostic significance (13, 14, 26–33). P120 has been indicated as a prognostic marker in breast cancer (15). This study suggests that P120 is associated with proliferative properties of human breast cells. However, the possibility cannot be ruled out that P120 is involved in other biological properties of breast cancer cell growth. The specific function of P120 remains unknown. Preliminary studies indicate that P120 may be involved in the initial phases of ribosomal biogenesis (12). It has long been known that increased ribosomal biogenesis is necessary for the progression from G1 to S-phase and is correlated with mitotic events (34).

In summary, the present study indicates that the level of P120 expression is positively correlated with the proliferation rate and that P120 expression is directly involved in the proliferation process.

Fig. 5. Decreased amount of P120 protein in P120 antisense oligodeoxynucleotide-treated MCF-7/AZ cells measured by "P-RT-PCR. A, silver-stained PAGE protein pattern; B, Western blot. Lane 1, control without treatment; lane 2, cells treated with nonsense oligomer; lane 3, cells treated with antisense oligomer. Cells were plated, treated, and harvested as described in the legend of Fig. 5. Three wells were prepared and combined for each treatment. Total RNA was prepared from each sample as described in "Materials and Methods." Absorbances of RNA solution at 260 nm were equalized after extractions with phenol-CHCl3. An equal amount of glycogen was added to each as carrier before the RNA precipitation. Two different concentrations from all four samples were used for cDNA synthesis in RT-PCR. cDNA synthesis, amplifications, and product analyses were carried out as described in "Materials and Methods."

Fig. 6. Decreased amount of P120 mRNA in P120 antisense deoxyribonucleotide-treated MCF-7/AZ cells measured by 32P-RT-PCR. A, 1 μg total RNA; B, 0.5 μg total RNA used for cDNA synthesis. Lane 1, negative control for PCR (glycogen without RNA); lane 2, control cells without treatment; lane 3, cells treated with nonsense oligomer; lane 4, cells treated with antisense oligomer. Cells were plated, treated, and harvested as described in the legend of Fig. 5. Three wells were prepared and combined for each treatment. Total RNA was prepared from each sample as described in "Materials and Methods." Absorbances of RNA solution at 260 nm were equalized after extractions with phenol-CHCl3. An equal amount of glycogen was added to each as carrier before the RNA precipitation. Two different concentrations from all four samples were used for cDNA synthesis in RT-PCR. cDNA syntheses, amplifications, and product analyses were carried out as described in "Materials and Methods."

![Fig. 5. Decreased amount of P120 protein in P120 antisense oligodeoxynucleotide-treated MCF-7/AZ cells. A, silver-stained PAGE protein pattern; B, Western blot. Lane 1, control without treatment; lane 2, cells treated with nonsense oligomer; lane 3, cells treated with antisense oligomer. In a well of a 24-well plate, 23,000 cells were plated. Three days later, antisense and nonsense oligomers in a concentration of 100 μM were added. On the fifth day medium was discarded, and viable cells were collected, counted, subjected to lysis, and loaded onto polyacrylamide gel as described in the legend of Fig. 2.](cancerres.aacrjournals.org)
REFERENCES


Effect of Nucleolar P120 Expression Level on the Proliferation Capacity of Breast Cancer Cells

Anna Fonagy, Carol Swiderski, Alexander M. Ostrovsky, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/54/7/1859

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.