E1A Specifically Enhances Sensitivity to Topoisomerase IIα Targeting Anticancer Drug by Up-Regulating the Promoter Activity

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Abstract

DNA topoisomerases I and II (topo I and II) are nuclear enzymes involved in cellular replication and are targets for several anticancer drugs. We showed previously that E1A gene transfer enhanced the sensitivity of Ewing's sarcoma cells to the topo IIα targeting agents etoposide and Adriamycin in vitro and in vivo. To determine whether this effect was specific for topo IIα, we investigated the effect of E1A gene transfer on cell sensitivity to agents that target topo I and IIβ. Transfecting TC71 human Ewing's sarcoma cells with an adenoviral vector containing the E1A gene enhanced their sensitivity to the topo IIα targeting agents etoposide (16-fold) and Adriamycin (8-fold). By contrast, E1A gene transfer did not affect cellular sensitivity to either amsacrine or camptothecin. Western blot analysis indicated that topo IIα protein levels increased 3.1-fold after E1A gene transfer, but topo I and IIβ protein levels did not change. A plasmid containing topo IIα gene promoter with luciferase reporter gene was constructed to determine the effects of E1A gene transfer on the activity of the topo IIα promoter. E1A increased the activity of the topo IIα gene promoter by 3.5-fold relative to that of cells transfected with Ad-β-gal. These results suggest that elevated topo IIα protein levels and enhanced sensitivity to topo IIα targeting agents were secondary to a direct effect of E1A on the topo IIα promoter. Combining E1A gene therapy with topo IIα targeting anticancer drugs may therefore have therapeutic benefit by increasing tumor cell sensitivity.


Introduction

Topoisomerases are nuclear enzymes that control DNA topology, cell differentiation, and cell replication. Many anticancer drugs stabilize the covalent complex formed between DNA and the topoisomerase enzymes, thereby initiating a biochemical cascade leading to cell death (1, 2). Resistance to chemotherapy has been shown to involve several different mechanisms, including alteration of topoisomerases (3-5). Topoisomerases have several different isofoms [i.e., topoisomerase I, IIα, and IIβ (topo I, IIα, and IIβ)]. Camptothecin and topotecan target topo I, etoposide (VP-16) and Adriamycin target topo IIα, and amsacrine targets topo IIβ (6, 7). We showed previously that E1A gene transfer into human Ewing's sarcoma cells increased cellular sensitivity to VP-16 and Adriamycin in vitro and in vivo (8, 9). This increased sensitivity was shown to be secondary to an increase in topo IIa expression. To ascertain whether this effect on topo IIα was specific and to further determine the mechanism responsible for enhanced topo IIα expression, we investigated the effect of the E1A gene on cell sensitivity to topo I and IIβ targeting agents and on the activity of the topo IIα promoter. We found that E1A gene transfer into TC71 human Ewing's sarcoma cells enhanced their sensitivity to topo IIα targeting drugs but not to agents that target topo I or IIβ. Topo IIα protein expression and the activity of the topo IIα promoter were also increased by E1A gene transfer. These results suggest that in addition to acting as a transcriptional repressor of HER-2/neu the E1A gene may act as a transcriptional promoter of topo IIα.

Results

Effect of E1A on the Sensitivity of TC71 Cells to Agents That Target Topo IIα, IIβ, or I

Our first step was to confirm that E1A sensitized TC71 cells to the topo IIα targeting agents VP-16 and Adriamycin. Transfection with the E1A gene increased cellular sensitivity to VP-16 by 16-fold (IC50, 90 nmol/L versus 1.5 μmol/L), whereas transfection with Ad-β-gal did not alter cell sensitivity (IC50, 1.3 μmol/L; Fig. 1A). E1A gene transfer also enhanced the sensitivity of TC71 cells to Adriamycin (IC50, 12 versus 100 nmol/L; Fig. 1B). By contrast, E1A transfer did not affect cellular sensitivity to amsacrine (a topo IIβ targeting agent) or camptothecin (a topo I targeting agent; Fig. 1C and D). Results for all drugs are summarized in Table 1. The IC50 for amsacrine was 2.1 versus 2.0 μmol/L with E1A and 3.0 versus 2.5 μmol/L with E1A for camptothecin.

Effect of E1A on Topo Iα, Iβ, and I Protein Expression

Western blotting analyses indicated that topo Iα protein levels increased by 3.1-fold after transfection with E1A (Fig. 2, top). In contrast, transfection with Ad-β-gal did not affect topo Iα protein expression. Transfection with E1A did not affect topo Iβ (Fig. 2, middle) or topo I (Fig. 2, bottom) protein levels compared with cells transfected with Ad-β-gal (Fig. 2).
These results suggest that the E1A gene specifically increased cellular topo IIα protein levels and that this effect led to their sensitivity to the topo IIα targeting drugs VP-16 and Adriamycin.

**Effect of E1A on Topo IIα Promoter Activity**

After finding that E1A gene transfer up-regulated topo IIα expression and protein production, we next investigated the effects of the E1A gene on the topo IIα promoter by using pGL-topo IIα-557, a vector containing the topo IIα gene promoter with a luciferase reporter gene. Transfection with adenoviral vector containing the E1A gene (Ad-E1A) increased the luciferase activity by 3.5-fold compared with Ad-β-gal.

Following treatment with Cdk2/cyclin inhibitory peptide II, which inactivated E2F-1 transcript factor function (10), Ad-E1A did not significantly alter the topo IIα promoter activity (Fig. 3, top). We verified the specificity of this activity by testing the effect of E1A on the Fas promoter in cells transfected with FPR-Fas-Luc. Treatment of those cells with Ad-E1A did not change Fas promoter activity. Transfer of the IL-12 gene using Ad-IL-12 served as our positive control (11) and resulted in increased Fas promoter activity (Fig. 3, bottom).

**Discussion**

In the present study, we showed that E1A gene transfer specifically enhanced the sensitivity of human Ewing’s
sarcoma cells to the topo IIα targeting drugs VP-16 and Adriamycin. 

Table 1. Effects of E1A on Cell Sensitivity to VP-16, Adriamycin, Amsacrine, and Camptothecin

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 for drug alone (mol/L)</th>
<th>IC50 for drug with Ad-E1A (mol/L)</th>
<th>Sensitivity enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP-16</td>
<td>1.5</td>
<td>90</td>
<td>16-fold</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>100</td>
<td>12</td>
<td>8-fold</td>
</tr>
<tr>
<td>Amsacrine</td>
<td>2.1</td>
<td>2.0</td>
<td>1.1-fold</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>4.0</td>
<td>4.5</td>
<td>1.2-fold</td>
</tr>
</tbody>
</table>

NOTE: TC71 cells were incubated with various concentrations of drugs. Cell cytostasis was quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described in Materials and Methods. Drug sensitivity enhancement was calculated by dividing the IC50 for drug alone by the IC50 for drug with Ad-E1A.

overexpress HER-2/neu by virtue of its ability to also up-regulate the topo IIα promoter, leading to increased topo IIα expression and increased topo IIα protein production, thereby increasing the drug target. Presumably, these effects will in turn enhance tumor sensitivity to topo IIα targeting agents, such as Adriamycin and VP-16.

Materials and Methods

Cell Line

TC71 human Ewing’s sarcoma cells (kindly provided by Dr. T. Triche, University of Southern California, Los Angeles, CA) were cultured in Eagle’s modified essential medium with 10% fetal bovine serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 1× nonessential amino acid, and 2× MEM vitamin solution (Life Technologies, Inc., Grand Island, NY). This cell line was screened with a Mycoplasma Plus PCR Primer Set (Stratagene, La Jolla, CA) and found to be free of Mycoplasma.

Recombinant Adenovirus

Ad-E1A (kindly provided by Dr. M-C. Hung, Department of Molecular and Cellular Biology, The University of Texas M.D. Anderson Cancer Center, Houston, TX) is an adenovirus type 5–based vector that contains E1A but lacks E1B and E3 (15). The control adenovirus, Ad-5-gal, is an adenovirus type 5 that lacks E1A, E1B, and E3 but contains β-galactosidase. Both of these recombinant replication-deficient adenoviral vectors were propagated in human embryonic kidney 293 cells as described previously (20). The viruses were purified twice by cesium chloride gradient ultracentrifugation and then dialyzed and

FIGURE 2. E1A increased topo IIα protein expression but not that of topo IIβ or I. TC71 cells were treated with PBS (lane 1), Ad-E1A (lane 2), or Ad-β-gal (lane 3) for 48 hours. Cellular protein was extracted and subjected to electrophoresis on SDS-PAGE gel. Expression of topo IIα (top), topo IIβ (middle), and topo I (bottom) proteins was detected by Western blot using specific antibodies. Densitometric analysis of each band was normalized with a β-actin loading control. Relative expression levels were calculated in comparison with the protein levels in the TC71 control cells.
Relative luciferase activity was calculated in comparison with that of control cells. Columns, mean of three independent experiments; bars, SD.

**Topo Ix Promoter Vector Construction**

The topo Ix promoter vector pGL-topo Ix-557 (kindly provided by Dr. D.P. Suttle, University of Tennessee Health Science Center, Memphis, TN) was created by subcloning the PCR product containing the topo Ix promoter into a pGL2 basic vector linking upstream of the luciferase gene. Plasmid DNA was purified with the Qiagen plasmid purification kit (Valencia, CA) and confirmed by restriction enzyme analysis (22). The Fas promoter FPR-Fas-Luc vector (23) used to verify the specificity of the effect on the topo Ix promoter was kindly provided by Dr. L.B. Owen (University of California, Riverside, CA).

**Acknowledgments**

We thank Dr. D.P. Suttle for providing the topo Ix promoter vector and M-C. Hung for the Ad-E1A vector.

**References**


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