Identification of New Drug Sensitivity Genes Using Genetic Suppressor Elements: Protein Arginine N-Methyltransferase Mediates Cell Sensitivity to DNA-damaging Agents

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ABSTRACT

Genetic suppressor elements (GSEs) are cDNA fragments encoding either truncated proteins, acting as dominant-negative mutants, or inhibitory antisense RNA segments counteracting with the gene from which they are derived. To identify genes controlling the cell response to cytotoxic agents, a normalized retroviral library of randomly fragmented cDNAs from Chinese hamster cell line DC-3F was screened for GSEs conferring resistance to the topoisomerase II inhibitor 9-OH-ellipticine. From 218 cDNA fragments isolated, 11 functional GSEs, corresponding to at least 8 independent genes, were selected. The gene corresponding to the most abundant GSE encodes two proteins, p77 and p82, highly homologous to proteins detected in various species and carrying the sequence motifs characteristic of the protein arginine N-methyltransferase family. Furthermore, a methylase activity was observed on myelin basic protein in immunoprecipitates of hemagglutinin-tagged p77 and p82. Therefore, p77 and p82 are the first identified members of a new protein arginine N-methyltransferase family. A decreased expression of these enzymes is associated with either resistance or hypersensitivity to a broad range of DNA-damaging agents. Our data indicate that down-regulation of these enzymes in the GSE-expressing cells would alter one or several steps downstream of the drug-target interaction in the drug-response pathway.

INTRODUCTION

Cell killing by cytotoxic agents is a complex process, regulated by many genes (1, 2). The outcome of cell treatment with a cytotoxic drug is determined by the balance between the activity of DR1 genes, of which the expression is required to inhibit certain steps in the drug activity pathway, and DS genes, the function of which is essential to the drug-mediated cell killing. DR phenotype could be either dominant, if it is associated with the expression of a DR gene, or recessive, if it results from inactivation of a DS gene (3). Much progress has been made in the identification of the dominant DR genes (1) that can be identified by positive expression selection. Analysis of recessive DS genes was technically more challenging and was made amenable by the development of the GSE approach (4, 5). GSEs are short cDNA fragments encoding peptides acting as dominant inhibitors of protein function or antisense RNAs inhibiting gene expression. Because GSEs behave as dominant selectable markers for the phenotype associated with the repression of the gene from which they are derived (4), they are well suited for the identification of DS genes. The feasibility of this approach was first demonstrated by isolation of GSEs conferring etoposide resistance from cDNA fragments of a known DS gene, encoding topoisomerase II (6). It was then extended to identification of a new DS gene, kinesin heavy chain, by screening random fragment library of total cellular cDNA (5). Here we applied this strategy to identification of new genes determining cell sensitivity to DNA topoisomerase II inhibitors.

As a model, we used the Chinese hamster lung fibroblast cell line DC-3F and the mutant DC-3F/9-OH-E, selected previously for resistance to the DNA topoisomerase II inhibitor 9-OH-E, that acquired multiple genetic alterations contributing to DR and resulting in various phenotypic changes (7–10). A normalized GSE library was prepared from DC-3F cells and screened for GSEs conferring 9-OH-E resistance. Eleven functional GSEs, corresponding to at least eight independent genes, were isolated. The gene corresponding to the most abundant GSE was found to encode proteins which belong to the protein arginine N-methyltransferase family (PRMT). A decreased expression of this gene was found to be associated with either resistance or hypersensitivity to various DNA-damaging agents.

MATERIALS AND METHODS

Preparation of a Normalized cDNA Fragment Library. A normalized library of random cDNA fragments of ~2.5 × 107 clones, inserted in the Clal site of the retroviral plasmid pLNCX (11), was prepared from DC-3F cells as described previously (5).

Library Transduction and 9-OH-ellipticine Selection. The library was packaged by transfecting BOSC 23 cells (20 60-mm plates) with 160 μg of library DNA (12). The viral suspension (2 × 105 IU/ml), collected 36 h later, was used to infect DC-3F/cI23 (20 100-mm plates), after treatment with DEAE dextran (20 μg/ml) for 20 min. DC-3F/cI23 are DC-3F cells made sensitive to retrovirus infection by transfection with the pJET plasmid, which carries an ectopic retrovirus receptor gene (13). Infection was repeated 12 h later. Under these conditions, ~20% of the cells were infected. Twenty-four h after the second infection, cells (4 × 105) were replated (3 × 105/100-mm dish) and treated 20 h later with 9-OH-E (0.075 μg/ml, 3 h). After removal of the drug, the surviving clones were allowed to grow for 8–10 days. Cells from each dish were harvested for DNA extraction, and PCR amplification and recloning of the proviral inserts (5). The following oligonucleotides were used as PCR primers: 5'-GCCCAAGCCTTGGT-AAACTCGTTGGATG-3' (sense) and 5'-ATGCGGTATCAAGACTGTGCGGAA-CCTAC-3' (antisense). The sequence of the sense primer was designed to eliminate the ClaI site. However, in this process one ATG codon was also altered. PCR products were digested by HindIII and ClaI, and cloned in the corresponding cloning sites of pLNCX in the same orientation as in the original clones, thus constituting a second library that is supposedly enriched in fragments conferring DR. This library was delivered to fresh target cells and subjected to a second round of selection. Surviving cells were collected as 26 independently selected populations and frozen.

Identification of GSEs Conferring 9-OH-E Resistance. After thawing and overnight growth, cells from one vial were plated in 100-mm plates at 1
or 2 × 10⁵ cells/plate, and treated with 9-OH-E (0.075 μg/ml, 3 h) 24 h later. The surviving clones were picked individually and grown for genomic DNA extraction. After PCR amplification with the pfu DNA polymerase (Stratagen), inserts were purified and sequenced. The ability of selected inserts to confer resistance to 9-OH-E was tested individually after cloning in pLNCX (pCR). Above that limit, the GSEs were scored as follows: 2-fold increased resistance; 3-fold: 2.5-fold; and >5-fold: +++.  

**Northern and Southern Blot Analyses, cDNA Cloning, and DNA Sequencing.** These procedures have been described previously (14). For quantititative intensity comparisons, bands were analyzed with the Bioprofil software (Vilber-Lourmat, Torcy, France).

**Construction of the pSV-p82/p77-IRES-neo Vector.** This vector was used for transfection of the 2.3 kb CDNA into DC-3F/9-OH-E cells and selection of clones stably expressing p82/p77. A fragment containing the encephalomyocarditis virus IRES and the neomycine selection marker was excised from the pLXIN vector (Clontech, Palo Alto, CA) by digestion with XhoI and Nhel. This fragment (blunt-ended) was ligated to pcDNA3.1 (Invitrogen, Groningen, the Netherlands) digested with XhoI (blunt-ended). From this vector, named pCMV-IRES-neo, a fragment containing the vector polylinker associated with the IRES-neo group was excised by digestion with Pmel and ligated to the plasmid pSVSport1 (Life Technologies, Inc.) digested with PstI (blunt-ended). The 2.3 kb cDNA was inserted in this vector polylinker, between the EcoRI and NolI sites, to give the final construct pSV-p82/p77-IRES-neo.

**In Vitro Transcription Assay.** *In vitro* transcription-translation of cDNAs, inserted in pBlueScript plasmids, was carried out using the “TNT coupled reticuiofus T3 system” from Promega, following manufacturer’s instructions, in the presence of [³⁵S]methionine (1175 Ci/mmol; NEN, Boston, MA). After fractionation by SDS-PAGE (10%) and treatment of the gel with Amplify reagent (Amerham), the reaction products were detected by fluorography at ~70°C for 30 min.

**Anti-GSTp77 Antibody.** Detailed plasmid construction, Escherichia coli expression, and purification of the fusion protein GSTp77 protein will be described elsewhere. A polyclonal rabbit antisera against GST-p77 fusion protein was prepared by Eurogentec Bel S.A. (Herstal, Belgium).

**Preparation of Cells Extracts and Western Blot Analysis.** Preparation of cell extracts, protein fractionation by SDS-PAGE, and immunoblotting conditions have been described previously (15). Proteins were transfected on Hybond-enhanced chemiluminescence nitrocellulose membrane (Amersham manufacturer) for 1 h at 1 A in 20 mM sodium phosphate buffer (pH 6.8). The primary rabbit antibody, anti-GSTp77, was used at 1:2000 dilution, and the secondary antibody, antirabbit horseradish peroxidase conjugated (Amersham Pharmacia Biotech) 20 μg of MBP (Sigma; Ref. 17) were added. After incubation at 30°C for 30 min, the reaction was arrested by adding 50 μl of 2x sample loading buffer and 5 min boiling.

**RESULTS**

**Characterization of GSEs Confering Resistance to 9-OH-E.** DC-3F/cl23 cells were infected with the GSE library enriched previously through one additional run of selection. Control cells were transfected with insert-free pLNCX, and both populations were submitted to a mild selection with 9-OH-E (0.075 μg/ml, 3 h). The survival of cells infected with library-derived viruses was at least three times higher than that of the control, as judged by the number of colonies (Fig. 1A). Twenty-six independently selected populations were stored in liquid nitrogen. After thawing, 6 of these populations were again treated with 9-OH-E (0.075 μg/ml, 3 h), and 345 resistant clones were individually picked and analyzed. This represents approximately 25–30% of the surviving clones for each population. PCR amplification showed that 60% of them contained at least one GSE (Fig. 1B). DNA fragments (218) were isolated. The properties of 64 of them, which were artifactual composite sequences, will not be shown. From the remaining 154 DNA fragments, 14 different sequences, numbered I to XIV, were identified (Table 1). GSE I and GSE II were strongly selected for because they represented 74% and 14% of these fragments, respectively. GSE VII and GSE XI, detected four times, were always alone, as were GSEs IX, X, XII, and XIV, detected once. GSEs III, IV, V, VI, VII, and XIII, also detected once, were associated with GSE I or with a composite fragment containing GSE I. Each of these 14 fragments was recloned into pLNCX, in the same position and orientation as in the original plasmid, and tested for their ability to render DC-3F/cl23 cells resistant to 9-OH-E. Eleven of them were identified as functional GSEs, able to induce 9-OH-E resistance (Fig. 1C). Three of them, GSEs IV, IX, and XIV, were unable to induce significant resistance. GSEs IX and XIV are likely inactive fragments present by chance in surviving cells. Whether GSE IV is able to increase the resistance level in GSE I-containing cells remains to be determined. Table 1 shows that at least 8 of these GSEs recognize different mRNAs, indicating that they correspond to at least 8 different genes.
was apparently decreased. However, Fig. 2 shows that expression of GSE I confers to DC-3F/cl23 cells a 5–10-fold resistance to various DNA-damaging agents, including topoisomerase II inhibitors (9-OH-E, S16020–2, and etoposide) and cisplatinum. In contrast, the sensitivity of these cells to the protein kinase inhibitor staurosporine was not altered.

We also observed that GSE I expression was associated with an increased sensitivity to some other DNA-damaging drugs. To additionally demonstrate this effect, the GSE I-transduced cells were submitted to an additional 9-OH-E selection in the same conditions as above. In this process, most noninfected cells were eliminated, and we additionally selected cells carrying the most efficient GSEs. Resistance to 9-OH-E was then increased up to ~20-fold (Fig. 2B). Fig. 2B shows that the sensitivity of these cells to UV radiations, bleomycin, which induces the formation of DNA single- and double-strand breaks, and the topoisomerase I inhibitor camptothecin was markedly increased.

**Effect of GSE I on Cleavage Complex Formation.** The diversity of GSE I effects on cell sensitivity to various agents makes it unlikely to interfere with a specific step in 9-OH-E mechanism of action. 9-OH-E, as well as most topoisomerase II inhibitors, kills cells by increasing the number of covalent enzyme-DNA cleavage complexes present on the genome at a given time, through formation of a topoisomerase-drug-DNA ternary complex (20). GSE I expression should not alter the formation of cleavage complexes in drug-treated cells, which can be determined by measuring the DNA alkaline elution rate in nondeproteinizing conditions (18). Cells were treated either with 9-OH-E (1 µg/ml) or VP-16 (10 µm) for 3 h. In these conditions, both drugs killed ~90% of the control DC-3F/cl23 cells; whereas survival of cells expressing GSE I was approximately 5–6-fold higher (Fig. 2A). Fig. 3 shows that, despite the differences of toxicities, expression of GSE I did not significantly alter the rate of DPC formation either in the controls or in drug-treated cells. After conversion of the DPC in rad equivalent (18), the difference between GSE I-expressing cells and controls treated with 9-OH-E was <20%, which for this technique is not significant. After VP-16 treatment, there was no difference at all. These results, which are consistent with our expectation, show that GSE I did not change the formation of the topoisomerase-drug-DNA complex, thus implying that the cellular concentrations of these complex components were not modified. In other words, GSE I has no effect on either drug cellular accumulation or topoisomerase II gene

Although sequence analysis revealed a high degree of similarity between GSE sequences and sequences reported previously (Table 1), these comparisons did not allow any unambiguous gene identification. GSE I, as the most abundant element, was picked for additional detailed analysis.

### Table 1 Properties of selected GSEs

<table>
<thead>
<tr>
<th>GSE</th>
<th>Size (bp)</th>
<th>Frequency</th>
<th>Resistance</th>
<th>Effect</th>
<th>mRNA length (kb)</th>
<th>Match</th>
<th>Score</th>
<th>E value</th>
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<tr>
<td>I</td>
<td>234</td>
<td>114</td>
<td>+ ++</td>
<td>Antisense</td>
<td>3.7–2.8</td>
<td>n cDNA</td>
<td>153</td>
<td>4.10^{-33}</td>
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<tr>
<td>II</td>
<td>194</td>
<td>22</td>
<td>+ ++</td>
<td>Sense</td>
<td>3.6</td>
<td>n</td>
<td>56</td>
<td>6.10^{-4}</td>
</tr>
<tr>
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<td>193</td>
<td>1</td>
<td>+ ++</td>
<td>Antisense</td>
<td>5.8</td>
<td>m Myg 1</td>
<td>1</td>
<td>6.10^{-5}</td>
</tr>
<tr>
<td>IV</td>
<td>180</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>181</td>
<td>1</td>
<td>+</td>
<td>N.D.</td>
<td>6–5.1–4.3</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>193</td>
<td>1</td>
<td>+</td>
<td>N.D.</td>
<td>N.D.</td>
<td>m Gamm 1</td>
<td>86</td>
<td>6.10^{-15}</td>
</tr>
<tr>
<td>VII</td>
<td>206</td>
<td>4</td>
<td>+++</td>
<td>N.D.</td>
<td>N.D.</td>
<td>/</td>
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<td></td>
</tr>
<tr>
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<td>+</td>
<td>N.D.</td>
<td>5–3.2</td>
<td>m chr 11</td>
<td>54</td>
<td>2.10^{-6}</td>
</tr>
<tr>
<td>IX</td>
<td>170</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td>/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td>185</td>
<td>1</td>
<td>+</td>
<td>Sense</td>
<td>4.5–3.2</td>
<td>n</td>
<td></td>
<td></td>
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<tr>
<td>XI</td>
<td>243</td>
<td>4</td>
<td>+</td>
<td>N.D.</td>
<td>N.D.</td>
<td>n</td>
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<tr>
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<td>n</td>
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<tr>
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<tr>
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<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td>/</td>
<td></td>
<td></td>
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</tbody>
</table>

\textsuperscript{a} Number of clones in which each GSE was found.

\textsuperscript{b} Ability of each GSE to confer 9-OH-E resistance to DC-3F cells was scored as positive when the number of surviving clones was at least twice as much as that in the control (CD-3F cells infected with insert-free vector).

\textsuperscript{c} Size of the mRNAs detected with each GSE by Northern blot analysis of a poly(A)-RNA library.

\textsuperscript{d} Homology of GSEs sequences with GenBank data, scores and E values were determined with the Blast algorithm (Altschul et al., 1997).

\textsuperscript{e} Definition, definition of database best match, h, human; n, no significant match; m, murine.

\textsuperscript{f} Score (in bits).

\textsuperscript{g} Statistical significance.
expression. Immunoblotting analysis confirmed that GSE I had no effect on topoisomerase II expression (data not shown). GSE I should then be acting downstream of the drug-interaction step.

Cloning of the cDNAs Corresponding to GSE I. GSE I hybridized with two transcripts with apparent sizes of ~3.7 and 2.8 kb on Northern blots of polyadenylated RNAs from DC-3F/cl23 cells (Fig. 4A, part 1). The same two transcripts were detected in mRNA from cells expressing GSE I, but their relative abundance was different: quantitative densitometry of hybridization signals, normalized according to actin loading control, showed that whereas the 2.8 kb RNA was equally represented in both preparations, the 3.7 kb transcript was 2–3-fold less abundant in the cells expressing GSE I (Fig. 4A, part 2). Two additional transcripts revealed by GSE I probe in the cells expressing GSE I (4 and 3.5 kb) are likely to represent RNAs transcribed from the pLNCX vector.

GSE I-corresponding cDNAs were isolated by plaque hybridization from the AZAP1cDNA library made from DC-3F cells (14). Eleven clones carrying inserts of 2.3, 2.5, and 3.2 kb, were identified (Fig. 4B). Sequence analysis showed that the shortest 2320 bp-long cDNA (Fig. 5A) contains two ORFs, encoding 729 amino acids and 692 amino acids and located between 40 and 2227 nucleotides, and 151 to 2227 nucleotides, respectively. The 2510 bp-long cDNA, containing an ORF encoding 655 amino acids from position 466 to 2431, differs from the 2.3-kb cDNA by two additional sequences (32 and 153 nucleotides) located upstream of the ORF. The longest 3214 bp-long cDNA contains an ORF from position 171 to 2247 encoding 692 amino acids. It differs from the previous cDNA clone by additional sequence (927 bp) extending from the stop codon to the end of the polyadenylic acid tail. In their coding regions, all of the three cDNAs
display a complete sequence identity. GSE I-encoded antisense RNA corresponds to a sequence located in the 5′ region of the ORF.

Analysis of Translation Products from GSE I Recognized mRNAs. In the in vitro transcription-translation assay (Fig. 5b), the 2.3-kb cDNA gave rise to two proteins with the expected molecular weights of Mθ, 82,000 and 77,000 (p82 and p77). There was no protein made from the 2.5-kb cDNA, likely because the first AUG codon is not in a favorable context to initiate translation (21). As expected, a single protein of Mθ, 77,000 was synthesized from the 3.2-kb cDNA, presumably initiated from the second initiation codon (position 171). The ORF from the first ATG (position 40) is interrupted by the 32-bp insertion (Fig. 3). Sequence comparisons showed that p77 is a truncated form of p82.

Both p77 and p82 were detected by Western blot in extracts made from DC-3F cells. The abundance of both proteins was approximately 2–3-fold decreased in GSE I-expressing cells (Fig. 6). A similarly decreased expression of p77 and p82 was observed in DC-3F/9-OH-E as compared with DC-3F cells (Fig. 6). An additional band corresponding to a Mθ, 69,000 protein was detected in cell extracts from DC-3F/9-OH-E cells. The origin of this protein is presently unknown. All of these results indicate that a decreased p77/p82 expression is associated with a decreased sensitivity to 9-OH-E.

Overexpression of p82 in DC-3F/9-OH-E Cells. From these results, we expected that an increased expression of p77/p82 should reverse the cell resistance to 9-OH-E. To confirm this hypothesis, DC-3F/9-OH-E cells were transfected with the 2.3-kb cDNA. Fig. 7a shows that, among 9 clones tested by Western blot analysis, a strong overexpression of p82 was detected in 3 of them, clones 4, 9, and 12. p77 was also expressed in these clones, but at a much weaker level. To determine their drug sensitivity, cells from clones 4, 9, and 11 were treated with 9-OH-E at 2.5 μg/ml for 3 h, and surviving cells were allowed to grow for 5 days before counting by crystal violet staining as described previously (22). In these conditions, control cells (two DC-3F/9-OH-E clones independently selected after transfection with empty vectors) grew normally. In contrast, Fig. 7b shows that the survival of cells from clones 4, 9, and 12 was reduced to 20–25% of that of the control. Identical results were observed after transfection of DC-3F/9-OH-E cells with the 3.2-kb plasmid, which codes only p77 (data not shown). These experiments then confirmed that an increased expression of p77/p82 is associated with an increased sensitivity to 9-OH-E. Altogether, these results demonstrate that expression of p77/p82 modulates the cell sensitivity to 9-OH-E.

The hypersensitivity to camptothecin, induced by GSE I expression in DC-3F cells, was not observed in DC-3F/9-OH-E cells, which in fact are cross-resistant to this drug. Overexpression of p82 in clones 4 and 9 did not alter the resistance of these cells to camptothecin, and identical results were observed with two clones overexpressing p77 (data not shown). It is likely that the hypersensitivity phenotype, expected to be associated with the decreased expression of p77 and p82 in DC-3F/9-OH-E cells, is concealed by other gene alterations, which accumulated during the very long selection process that led to the isolation of these cells.

Sequence Homology between p77 or p82 and Protein Arginine N-Methyl Transferases. The amino acid sequences of p77 and p82, predicted from the nucleotide sequences, were compared with protein sequences in the GenBank databases. In initial searches, sequence alignments in the NH2-terminal regions of p77 and p82 revealed sequences that are highly homologous to sequence motifs that are conserved in the family of AdoMet-dependent PRMTs. These sequences, designated as motifs I, post-I, -II, and -III (23), are always found in the same order in the peptide sequence and are separated with comparable intervals (23, 24). Two other motifs, named double-E loop and THW loop, characterized by the presence of invariant amino acids in the loop sequences, form the enzyme active site (25). All of these common structural traits, required for PRMT activity, are also present in p77 and p82 (Fig. 8a). Additional searches, among sequences introduced more recently in the GenBank databases, led to the identification of protein sequences from Homo sapiens, Mus musculus, Drosophila melanogaster, and Caenorhabditis elegans, highly homologous to p77 and p82. For instance, the human sequence displays 83% identity with the complete cDNA-predicted p77 and p82 sequences (Fig. 8b). However, no biochemical activity for these proteins has been reported thus far.

Protein Methyl Transferase Activity in Immunoprecipitates of p77 and p82. Because sequence analyses indicated that p77 and p82 belong to a highly conserved group of proteins carrying an AdoMet-dependent protein methyltransferase activity, we determined whether these proteins could carry such an activity. DC-3F cells were trans-

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6 GenBank sequence accession numbers are: 2.3 kb cDNA, AF336043; 3.2 kb cDNA, AF336044; and 2.5 kb cDNA, AF 336045.
fected with insert-free pcDNA3-HA3 (control), pcDNA-HAp77, or pcDNA-HAp82 plasmids. Immunoprecipitates from cell extracts with the anti-HA antibody were incubated with MBP and [3H]AdoMet. The reaction mixtures were then fractionated by SDS-PAGE. Fig. 9 shows that there was no detectable transfer of radioactive methyl groups with immunoprecipitates from the control extract. However, incubation of MBP with immunoprecipitates from transfected cells expressing either p77 or p82 resulted in transfer of methyl groups from [3H]AdoMet to MBP, in proportion of the amount of p77 or p82 protein in the reaction mixture. These data indicated that p77 and p82 carry a PRMT activity.

DISCUSSION

The selection of GSEs has the unique capability to directly identify genes functionally related to a given phenotype. Using this approach to identify new genes involved in 9-OH-E sensitivity, 14 different sequences, most of them corresponding to independent genes, were
isolated. Eleven of these fragments were identified as functional GSEs able to individually confer resistance to 9-OH-E. These fragments tag at least 8 independent genes, the inactivation of which generate 9-OH-E resistance, illustrating the complexity of this phenotype. Approximately 30% of the 9-OH-E-resistant clones contained 2 or 3 fragments. Whether the properties of these GSEs are functionally additive will have to be examined. However, the development of resistance to drugs inhibiting multistep processes may be favored by GSE combinations as shown previously for the DNA replication inhibitor aphidicolin (26).

A puzzling observation was the very high frequency of GSE I recovery, which was present, either alone or in combination with another fragment, in ~50% of the selected clones. Recently, GSE I was again independently isolated from our cDNA fragment library after a selection for resistance to a prolonged exposure to a mixture of etoposide and cisplatinum. This observation is in agreement with the cross-resistance pattern induced by GSE I. However, in a previous work, Gudkov et al. (5) isolated three GSEs inducing resistance to etoposide, another topoisomerase II inhibitor. Two of them were derived from unknown genes, whereas the third encoded an antisense RNA for a kinesin heavy chain. None of these fragments corresponded to that isolated in this work. Different tissue origin, as well as technical conditions of library preparation and selection procedures, are expected to largely influence the representation of each GSE in different libraries.

The gene from which GSE I was derived was identified to a PRMT gene by sequence analysis and, more directly, by detection of this enzyme activity in cDNA-transfected cells. Presently known PRMTs share a common region of homology corresponding to the catalytic core domain, but differ from one another by NH2- and/or COOH-terminal extensions, outside of this domain. The mammalian PRMT1 and the yeast Hmt1, which are 353 and 348 amino acids in length, respectively, can be considered as essentially representing the catalytic core (27, 28). In p77 and p82, the catalytic core is located in the NH2-terminal half of the polypeptide chain, which displays a 28% sequence homology with PRMT1 and 26% with Hmt1, respectively. However, p77 and p82 markedly differ from other PRMTs by a unique COOH-terminal sequence of ~350 amino acids. However, amino acid sequences closely related to p77/p82, but corresponding to proteins of unknown biochemical functions, have been reported in *H. sapiens*, *M. musculus*, *D. melanogaster*, and *C. elegans*. p77 and p82 then appear as the first representatives of a new group of highly conserved proteins in the PRMT family.

In cells expressing GSE I, the abundance of p77 and p82 was 2–3 fold reduced. The amount of the 3.7-kb mRNA, coding for p77, was also decreased approximately in the same proportion. Because the 2.8-kb mRNA is much more abundant than the 3.7 kb in DC-3F cells, a similar reduction of the amount of this transcript might not be detectable. Nevertheless, these data indicated that a reduced PRMT expression is responsible for the GSE I-induced resistance phenotype. This conclusion is strongly strengthened by two additional observations: (a) 9-OH-E-resistant cells DC-3F/9-OH-E also contain reduced amounts of p77 and p82, indicating that down-regulation of p82/p77 expression may constitute a natural resistance mechanism in 9-OH-E-resistant cells selected by usual procedures; and (b) overexpression of p82/p77 in DC-3F/9-OH-E cells partially restored their sensitivity to 9-OH-E.

PRMTs catalyze the sequential transfer of methyl groups from AdoMet to the guanidino nitrogens of arginine residues in various substrates, including RNA binding proteins (29), transcription factors (30), nuclear matrix binding proteins (30), cytokines (31), and cytochrome c (32). The diversity of PRMT targets reflects their implication in a variety of cellular processes, such as transcriptional regulation (33), mitotic stimulation (16), or signal transduction (34). However, a function of PRMTs in the response to DNA damage has never been envisaged. The diversity of GSE I effects suggested that down-regulation of p82/p77 should alter one or several steps downstream of the drug-target interaction in the drug-response pathway. p77 and p82 are representative of a new group in the PRMT family, the physiological role of which is still unknown. The understanding of this function will require the identification of p77- and p82-specific substrates.

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CONTROL OF CELL SENSITIVITY TO DNA DAMAGE BY PRMT


Identification of New Drug Sensitivity Genes Using Genetic Suppressor Elements: Protein Arginine N-Methyltransferase Mediates Cell Sensitivity to DNA-damaging Agents

Laurent Gros, Charlotte Delaporte, Stéphane Frey, et al.


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