Induction of Apoptosis and Inhibition of Cell Proliferation by survivin Gene Targeting*

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Survivin is a new IAP apoptosis inhibitor expressed during development and in human cancer in vivo. The coding strand of the survivin gene was extensively complementary to that of effector cell protease receptor-1 (EPR-1), prompting the present investigation on the origin and functional relationship of these two transcripts. Southern blots of genomic DNA were consistent with the presence of multiple, evolutionarily conserved, EPR-1/Survivin-related genes. By pulsed field gel electrophoresis and single- and two-color fluorescence in situ hybridization, these were contained within a contiguous physical interval of 75–130 kilobases (kb) on chromosome 17q25. In Northern blots, a single strand-specific probe identified a 1.3-kb EPR-1 mRNA broadly distributed in normal adult and fetal tissues, structurally distinct from the 1.9-kb Survivin transcript expressed in transformed cell lines. Transient co-transfection of an EPR-1 cDNA potentially acting as a Survivin antisense with a lacZ reporter plasmid resulted in loss of viability of HeLa cells. In contrast, co-transfection of an antisense cDNA of intercellular adhesion molecule-1 or a sense-oriented Survivin cDNA was without effect. In stably transfected HeLa cells, SnzSO4 induction of an EPR-1 mRNA under the control of a metallothionein promoter suppressed the expression of endogenous survivin. This resulted in (i) increased apoptosis as detected by analysis of DNA content and in situ internucleosomal DNA fragmentation and (ii) inhibition of cell proliferation as compared with induced vector control transfectants. These findings suggest the existence of a potential EPR-1/survivin gene cluster and identify survivin as a new target for disrupting cell viability pathways in cancer.

Regulated inhibition of programmed cell death (apoptosis) preserves normal homeostasis and tissue and organ morphogenesis (1, 2). Aberrations of this process participate in human diseases and may contribute to cancer by abnormally prolonging cell viability with accumulation of transforming mutations (3). Recently, several apoptosis inhibitors related to the baculovirus iap gene have been identified in mouse, Drosophila, and human (4). Intercalated in TNF receptor signaling (5, 6) and NF-κB-dependent survival (7), IAP proteins contain two/three Cys/His baculovirus IAP repeats plus a carboxyl terminus RING finger and are thought to block an evolutionarily conserved step in apoptosis (6, 8–10). At least in the case of XIAP (8), this may involve direct inhibition of the terminal effector caspases –3 and –7 (11). A novel member of the IAP gene family, designated Survivin (12), was recently identified by hybridization screening of human genomic libraries with the cDNA of a factor Xa receptor, effector cell protease receptor-1 (EPR-1) (13). Unlike all other IAP proteins (4), Survivin contained a single baculovirus IAP repeat and no RING finger and was selectively expressed during development and in all the most common human cancers but not in normal adult tissues in vivo (12). Intriguingly, the Survivin coding strand was extensively complementary to that of EPR-1, thus suggesting a potential functional interaction between these two transcripts (12).

In this study, we sought to dissect the molecular relationship between EPR-1 and Survivin (12, 13) and its role in apoptosis inhibition. We found that EPR-1 and Survivin are encoded by structurally and topographically distinct messages potentially originating from a gene cluster at 17q25. Secondarily, down-regulation of Survivin by forced expression of EPR-1 increased apoptosis and inhibited growth of transformed cells.

EXPERIMENTAL PROCEDURES

Cells and Cell Cultures—Peripheral blood mononuclear cells were isolated from heparinized blood collected from normal informed volunteers by differential centrifugation on Ficoll-Hypaque (Amersham Pharmacia Biotech) at 400 × g for 22 °C and washed in phosphate-buffered saline, pH 7.4. The epithelial carcinoma HeLa cell line was obtained from American Type Culture Collection (Rockville, MD) and maintained in culture in complete growth medium (BioWhittaker, Walkersville, MD) supplemented with 10% fetal bovine serum (BioWhittaker) and 2 mM l-glutamine, according to the manufacturer’s specifications.

Chromosomal Location of an EPR-1/Survivin Locus—For fluorescence in situ hybridization, purified DNA from a Survivin P1 genomic clone (12) was labeled with digoxigenin dUTP (Amersham Pharmacia Biotech) by nick translation, combined with sheared human DNA, and hybridized to normal metaphase chromosomes derived from phytohemagglutinin-stimulated peripheral blood mononuclear cells in 50% formamide, 10% dextran sulfate, and 2× SSC. For two-color staining, biotin-conjugated probe D17Z1, specific for the centromere of chromosome 17, was co-hybridized with the digoxigenin-labeled P1 clone. Specific chromosomal staining was detected by fluoresceinconjugated anti-digoxigenin antibodies and Texas red avidin. Slides were counterstained with propidium iodide or DAPI for one- or two-color labeling, respectively. A total of 80 metaphase cells were analyzed with 89 cells exhibiting specific labeling.

Southern Hybridization—Human genomic DNA was extracted from HeLa cells, digested with EcoRI, BamHI, XbaI, or HindIII, separated on a 0.8% agarose gel and transferred to GeneScreen nylon membranes
(NEN Life Science Products). After UV cross-linking (Stratagene, San Diego, CA), the membranes were prehybridized with 100 μg/ml of denatured salmon sperm DNA (Promega Corp., Madison, WI) in 5× SSPE, 0.5% SDS, 5× Denhardt’s solution, and 0.1% sodium pyrophosphate at 65 °C in a roller hybridization oven (Hoefer Scientific, San Francisco, CA) for 4 h. Prehybridizations in other experiments were performed in low melting preparative agarose (Bio-Rad) at the concentration of 2× 10^6/220 μl block, and DNA was extracted by standard procedures. After block digestion with MluI or NotI, samples were separated by pulsed field gel electrophoresis on a 1% agarose gel for 20 h at 200 V with a pulse time of 75 s using a Bio-Rad CHEF DRII apparatus. After transfer to nylon membranes and UV cross-linking, hybridization with the EPR-1 cDNA and washes were carried out as described. In another series of experiments, a blot containing aliquots of genomic DNA isolated from several species (CLONTECH, San Francisco, CA) was hybridized with a 3′ 548-nt fragment of the EPR-1 cDNA, as described above.

**Northern Blots—**Multiple tissue blots of adult and fetal mRNA (CLONTECH, CA) were prehybridized with 100 μg/ml of denatured salmon sperm DNA (Promega) and hybridized with an EPR-1 single-strand specific probe (see below) in 5× SSPE, 10× Denhardt’s, 2% SDS, for 14 h at 60 °C. The membranes were washed twice in 2× SSC, 1% SDS for 30 min at 60 °C and once in 0.2× SSC at 22 °C before exposure for autoradiography. An EPR-1 specific single strand probe was generated by asymmetric polymerase chain reaction amplification of a 301-nt fragment of the EPR-1 cDNA generated by EcoRI (cloning site) and SacII digest and comprising the first 5′ 226 nt of the EPR-1 coding sequence plus 75 nt of the retained regulatory intron (13). The gel-purified fragment was mixed with 15 pmol dNTP (New England Biolabs, Beverly, MA), 7.5 pmol of dCTP, and 25 μCi of [α-32P]dCTP (Amersham Pharmacia Biotech) in 20 ml Tris HCI, 50 mM KCl, pH 8.4, 1.5 mM MgCl₂, plus 0.2 μg/ml of a SacII reverse EPR-1 primer 5′ TCCTG GCCGGG TCCCTCGCCCTGCTG and 2.5 units of Taq DNA polymerase (Life Science) in a total volume of 10 μl. 25 cycles of amplification were carried out with denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1 min. After centrifugation through a Sephadex G-50 spin column (Worthington Biochemical Corp, Freehold, NJ) at 14,000 × g for 5 min, the EPR-1 or Survivin probes were heated at 100 °C for 2 min and immediately added to the various hybridization reactions.

**Transient Transfections of Antisense Constructs—**A control antisense construct of intercellular adhesion molecule-1 was generated by polymerase chain reaction amplification of the full-length human intercellular adhesion molecule-1 cDNA (14) using oligonucleotides 5′-GATCTA-GACTGCTATGGCTCCCAGC-3′ and 5′-CCGCAAGGGTTCAGCAGG-GCGGCTCCTCCCTGCTG-3′ and 25 units of Taq DNA polymerase (Life Science) in a total volume of 10 μl. 25 cycles of amplification were carried out with denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1 min. After centrifugation through a Sephadex G-50 spin column (Worthington Biochemical Corp, Freehold, NJ) at 14,000 × g for 5 min, the EPR-1 or Survivin probes were heated at 100 °C for 2 min and immediately added to the various hybridization reactions.

**Generation of Inducible Survivin Antisense Transfectants—**The 708-nt Smal-EcoRI fragment of the EPR-1 cDNA (see above) was directionally cloned in the sense orientation in the mammalian cell expression vector pML1 (a gift of Dr. R. Pytel, University of California, San Francisco). The vector is derived from the episomal mammalian expression vector pcEP4 (15) and was carrierc by electroporation of a 7.2-kb HindIII-EcoRI fragment including the P1 genomic clone (16), and the Survivin antisense cassette with the MMT1 promoter, directing Zn²⁺-dependent expression of recombinant proteins in mammalian cells (15). 10 million HeLa cells were transfected with 10 μg of control pcDNA3 vector or the Survivin antisense by electroporation as described above. 48 h after transfection, cells were diluted, plated onto 100-mm diameter tissue culture dishes, and selected for 4 weeks in complete growth medium using 0.4 mg/ml hygromycin. Modulation of survivin expression in control cultures or Zn²⁺−induced antisense transfectants was carried by immunoblotting of detergent-solubilized cell extracts using 25 μg/ml aliquots of the affinity-purified antibody raised against the survivin sequence Ala²⁰-Ile⁴⁰, as described (12). In control experiments, Zn²⁺−induced vector control or survivin antisense transfectants were analyzed for modulation of Class I major histocompatibility complex by flow cytometry with monoclonal antibodies.

**Modulation of Apoptosis and Cell Growth in Inducible Survivin Antisense Transfectants—**Vector control or Survivin antisense transfectants were treated with 200 μM ZnSO₄ in 0% fetal bovine serum for 24 h at 37 °C following by in situ determination of apoptosis by internucleosomal DNA fragmentation (TUNEL). Briefly, cells were harvested and centrifuged at 800 × g for 10 min at 4 °C, the pellet was fixed in 10% formalin overnight, dehydrated, and embedded in paraffin blocks, and sections of 3–5 μm were put on high adhesive slides. Samples were treated with 20 μg/ml proteinase K for 15 min at 22 °C, washed in distilled water, quenched of endogenous peroxidase in 2% H₂O₂, in phosphate-buffered saline, and subsequently mixed with digoxigenin-labeled dUTP in the presence of terminal deoxynucleotidyl transferase followed by peroxidase-conjugated anti-digoxigenin antibody. Nuclear staining in apoptotic cells was detected with 3’, 3’-diaminobenzidine tetrahydrochloride dihydrate, according to the manufacturer’s instructions (ApopTag, Oncor, Gaithersburg, MD). For control experiments, the enzyme incubation step was omitted. Morphologic features of apoptotic cells (apoptotic bodies) under the various conditions tested were also analyzed by hematoxylin/eosin staining. For proliferation experiments, vector control or Survivin antisense transfectants at 2 × 10⁵ cells were transferred into 24-well culture plates (Costar) and induced with 200 μM ZnSO₄ in complete growth medium for 16 h at 37 °C, and cell proliferation was determined microscopically at 24 h intervals by direct cell count. Two independent clones of HeLa cell transfectants were used in these experiments with comparable results. In some experiments, analysis of DNA content in induced vector control or Survivin antisense transfectants in complete growth medium was carried out by propidium iodide staining and flow cytometry, as described above.

**RESULTS**

**Identification of an EPR-1/Survivin Locus—**A digoxigenin-labeled P1 genomic clone (~100 kb) containing all four exons of the survivin gene (12) specifically labeled a single region on the long arm of a group E chromosome by fluorescence in situ hybridization (Fig. 1A). In two-color staining with probe D17Z1 specific for the centromere of chromosome 17, the Survivin P1 clone reacted with the long arm of chromosome 17 with band 17q25 (Fig. 1A and B).

Probing human genomic DNA with the EPR-1 cDNA revealed several hybridizing bands (Fig. 2A). Of these, a ~7.5-kb XbaI, a 7.6-kb BamHI, and four HindIII fragments of ~15, 7.5, 6.4, and 3.7 kb, respectively (Fig. 2A, arrowheads), were not predicted from the complete restriction map of 14,796 nt of the survivin gene (12). In contrast, other bands of comparable intensity, including a 5.1-kb XbaI and a 7.1-kb BamHI fragment, or of stronger intensity, including fragments of 17.5-kb HindIII, 10.5-kb XbaI, 8.5-kb BamHI, and ~25-kb EcoRI (Fig. 2A), genuinely originated from the survivin gene (12). At variance with this complex hybridization pattern, pulsed field gel electrophoresis of high molecular mass human genomic DNA revealed only single EPR-1-hybridizing bands of ~75 and ~130 kb in MluI- or NotI-digested samples, respectively (Fig. 2B).
Finally, the EPR-1 cDNA strongly hybridized with several bands in genomic DNA from various mammalian species, with fainter signals in rabbit or chicken DNA (Fig. 2).

**Differential Tissue Distribution of EPR-1 and Survivin Transcripts**—Consistent with the size of the spliced EPR-1 message (13), a single strand EPR-1-specific probe detected a prominent ~1.3-kb EPR-1 mRNA band in most adult and terminally differentiated human tissues (Fig. 3, upper panel). Strong EPR-1 expression was observed in human pancreas, skeletal muscle, heart, and various hematopoietic cell types, including peripheral blood leukocytes, lymph node, and spleen (Fig. 3, upper panel). Consistent with the reactivity of an anti-EPR-1 antibody with fetal tissues (16), a 1.3-kb EPR-1 mRNA was also found prominently in fetal kidney and liver and less abundantly in fetal lung and brain (Fig. 3, lower panel). Control hybridization with an actin probe confirmed comparable loading of mRNA in the various fetal samples (Fig. 3). In contrast, a Survivin-specific single strand probe did not react with mRNA isolated from normal adult tissues (12), whereas it detected a prominent ~1.9-kb transcript plus a fainter 3.4-kb species in various transformed cell lines (not shown) and in agreement with previous observations (12).

**Effect of EPR-1 Expression on Apoptosis and Cell Proliferation**—Transient co-transfection of HeLa cells with an EPR-1 cDNA potentially acting as a Survivin antisense plus a lacZ reporter plasmid produced significant loss of viability in β-galactosidase-expressing cells (Fig. 4). In contrast, co-transfection of pcDNA3 vector alone, a sense-oriented Survivin construct, or a control antisense of intercellular adhesion molecule-1 cDNA did not affect HeLa cell viability under the same experimental conditions (Fig. 4). To determine more precisely the effect of regulated expression of EPR-1 on Survivin inhibition of apoptosis, HeLa cells were stably transfected with an EPR-1 cDNA under the control of a metallothionein-inducible promoter. In these experiments, ZnSO₄ induction of EPR-1 mRNA suppressed the expression of endogenous Survivin, as determined by immunoblotting with an anti-Survivin antibody (Fig. 5A). In

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**Fig. 1.** Chromosomal location of the EPR-1/Survivin locus. A dihydrogen-labeled human P1 genomic clone containing the entire survivin gene was incubated with metaphase chromosomes isolated from phytohemagglutinin-stimulated peripheral blood mononuclear cells in 50% formamide, 10% dextran sulfate, and 2 x SSC. The EPR-1-hybridizing gene was mapped in single-color labeling to the long arm of a group E chromosome (A, green staining) and located to band 17q25 (B, green staining) in two-color staining with probe D17Z1 specific for the centromere of chromosome 17 (B, red staining).

**Fig. 2.** Complexity and evolutionary conservation of EPR-1-related sequences. A, human genomic DNA was digested with the indicated restriction enzymes and hybridized with the EPR-1 cDNA. Radioactive bands indicated by arrowheads (7.6-kb BamHI, 7.5-kb XbaI, and four HindIII fragments of 15, 7.5, 6.4, and 3.7 kb) are not predicted by the map of the survivin gene (12). B, high molecular mass human genomic DNA was digested with the indicated restriction enzymes, separated by pulsed field gel electrophoresis, and hybridized with the EPR-1 cDNA. C, EcoRI-digested genomic DNA from the indicated species was hybridized with a 3’ 548-nt fragment of the EPR-1 cDNA. For all panels, molecular mass markers in kb are shown on the left.
In this study, we have shown that EPR-1 (13) and Survivin (12) are encoded by structurally and topographically distinct mRNA transcripts potentially originating from a gene cluster at 17q25. Secondly, constitutive or metallothionein induction of EPR-1, potentially acting as a Survivin antisense, down-regulated endogenous Survivin in transformed cells and resulted in increased apoptosis and inhibition of cell proliferation, even in the presence of optimal serum mitogen concentrations.

Among the regulators of programmed cell death (apoptosis), IAP proteins have recently attracted considerable attention for their ability to suppress an evolutionarily conserved step in apoptosis (4), potentially involving direct caspase inhibition (11). Deregulation of this pathway may also participate in human diseases, because inactivating mutations of neuronal

**FIG. 4. Effect of Survivin antisense expression on HeLa cell viability.** HeLa cells were transiently co-transfected with the EPR-1 cDNA potentially acting as a Survivin antisense or the various indicated constructs plus a lacZ reporter plasmid by LipofectAMINE. Cell viability in β-galactosidase-expressing cells was determined morphologically after a 48-h culture. Data are the means ± S.D. of at least two independent experiments.

in significant inhibition of cell proliferation, as compared with induced vector control cultures (Fig. 6A). 3 days after metallothionein induction, the number of vector control HeLa cell transfectants increased by 288% during optimal serum mitogen stimulation, as opposed to a 20% increase in Survivin antisense transfectants, under the same experimental conditions (Fig. 6A). The increased cell proliferation observed at later time intervals (days 4–5) in induced antisense transfectants may reflect heterogeneity in antisense expression with selective expansion of low expressing cells (Fig. 6A). The potential ability of Survivin to modulate apoptosis and cell proliferation under optimal concentrations of serum mitogens was further investigated. Analysis of DNA content in transiently transfected HeLa cells revealed a 2-fold increase in the fraction of apoptotic cells (sub-G<sub>1</sub> peak) in Survivin antisense transfectants as compared with vector control cells, under the same experimental conditions (Fig. 6B, M1 marker). This was also associated with a 15–20% decrease in the G<sub>2</sub>/M fraction in Survivin antisense transfectants, as compared with vector control cultures (Fig. 6B, M4 marker). In stable HeLa cell transfectants, zinc induction of Survivin antisense under optimal growth conditions produced a 1.4-fold increase in the sub-G<sub>1</sub> fraction and a ~20–36% reduction in the G<sub>2</sub>/M peak, as compared with induced vector control cultures (n = 2).

**DISCUSSION**

contrast and consistent with the expression of Survivin in transformed cell types, a single 16.5-kDa Survivin band was immunoblotted in metallothionein-induced HeLa cells transfected with the pML1 vector alone (Fig. 5A). In control experiments, metallothionein induction of EPR-1 mRNA did not affect the expression of Class I major histocompatibility complex molecules in HeLa cell transfectants, and no modulation of Survivin expression was observed in the absence of ZnSO<sub>4</sub> (not shown). Under these experimental conditions, antisense down-regulation of Survivin resulted in massive apoptosis in growth factor-deprived HeLa cells, as detected by in situ internucleosomal DNA fragmentation by the TUNEL system (Fig. 5B, panel 1). Specific nuclear staining was observed in 60–70% of metallothionein-induced, serum-starved HeLa cell transfectants, whereas induced vector control cultures did not stain with the digoxigenin-labeled dUTP probe (Fig. 5B, panel 3). No staining was observed in the absence of terminal deoxynucleotidyl transferase labeling (not shown). Hematoxylin/eosin staining confirmed the presence of numerous apoptotic bodies in ZnSO<sub>4</sub>-induced Survivin antisense transfectants, as compared with vector control HeLa cells (Fig. 5B, panels 2 and 4, arrowheads). The effect of antisense down-regulation of Survivin on HeLa cell proliferation was also investigated. As shown in Fig. 6A, suppression of endogenous Survivin resulted

**FIG. 3. Tissue distribution of EPR-1 mRNA.** Northern hybridization was carried out on multiple adult (top panel) and fetal (lower panel, A) tissue mRNA blots with an EPR-1-specific single strand probe. Lower panel, B, control hybridization with actin on human fetal tissues. Molecular mass markers in kb are shown on the left. PBL, peripheral blood leukocytes.
apoptosis inhibitory protein contributed to spinal muscular atrophy (9), and this molecule was cytoprotective against cerebral ischemia in vivo (17). More recently, this paradigm has been extended to cancer, with the identification of Survivin as a structurally unique IAP protein selectively expressed during development and in all the most common human cancers but not in normal adult tissues in vivo (12). Intriguingly, the survivin gene was identified by hybridization with the EPR-1 cDNA, and its coding sequence was found to be extensively complementary to that of EPR-1 (12), suggesting the possibility of apoptosis regulation by a potential interaction between these two transcripts, i.e. natural antisense (18–21).

**Fig. 5. Effect of metallothionein induction of EPR-1 mRNA on Survivin expression and apoptosis.** A, aliquots of HeLa cells stably transfected with the empty pML1 vector (Vector) or the EPR-1 cDNA potentially acting as a Survivin antisense (Antisense) were induced with 200 μM ZnSO₄, detergent-solubilized, and immunoblotted with the anti-survivin antibody. Molecular weight (×10⁻³) markers are shown on the left. B, the experimental conditions are as in A, except that serum-starved Survivin antisense transfectants (1 and 2) or vector control cells (3 and 4) were stained for internucleosomal DNA fragmentation by the ApopTag method (TUNEL) (1 and 3) or by hematoxylin-eosin (2 and 4). Arrowheads, apoptotic bodies. Magnification, ×400.

Here, Southern blots of human genomic DNA were consistent with the presence of multiple, evolutionarily conserved, EPR-1/Survivin-related genes, with several hybridizing fragments that could not be recapitulated by the complete physical map of 14,796 nt of the survivin gene (12). Despite this complex hybridization pattern, pulsed field gel electrophoresis and fluorescence in situ hybridization studies suggested the existence of a single EPR-1/Survivin locus spanning 75–130 kb on chromosome 17q25. Although mammalian genes transcribed in both directions have been described (22, 23), these data are more consistent with a model of separate genes encoding EPR-1 and Survivin, potentially arisen from duplication event(s) and clustered in relatively close proximity at 17q25 in a head-to-head configuration (24). The use of single strand-specific probes further demonstrated that EPR-1 and Survivin originated from two structurally different messages of 1.3 and 1.9 kb, respectively, expressed in a mutually exclusive fashion in adult and fetal tissues (12, 16). This is consistent with the heterogeneity of EPR-1 transcripts detected by conventional, double strand probes with hybridizing bands of 1.9, 3.4, and ~1.5 kb previously identified in EPR-1⁺ cells (25). Although it is currently
not known if these two messages actually interact in vivo, we found that metallothionein induction of an EPR-1 mRNA suppressed the expression of endogenous Survivin in transfected cells. Consistent with the anti-apoptosis properties of Survivin (12), this resulted in increased apoptosis and significant inhibition of cell proliferation. Although accentuated by serum mitogen withdrawal, HeLa cell apoptosis following antisense down-regulation of Survivin was also observed under optimal growth conditions and was associated with a reduced number of proliferating cells in the G2/M fraction. In these experiments, the use of a noncoding EPR-1 cDNA potentially acting as a Survivin antisense ruled out the possibility that inhibition of Survivin was due to protein interactions. It is also unlikely that ZnSO4 induction of the metallothionein promoter may exert an independent anti-apoptotic function, because this has been attributed to ZnCl2, at concentrations 5–10-fold higher than those used here (26).

The findings described here may have profound implications for cancer therapy, where antisense-based strategies have been postulated for inhibition of several proto-oncogenes (27). Specifically, antisense blockade of anti-apoptotic bcl-2 decreased survival of leukemic cells in vitro (28), reduced tumorigenicity of lymphoma cells in athymic mice (29), and provided, at least in some cases, a positive therapeutic response in patients with non-Hodgkin’s lymphoma (30). In this context and consistent with the data presented here, targeting Survivin may selectively increase the susceptibility of cancer cells to apoptosis-based treatment and reduce their overall growth potential. In addition to inhibiting cell viability pathways distinct and complementary with those of bcl-2 (11), suppression of Survivin by an endogenous EPR-1 transcript potentially acting as a natural antisense may overcome the drawbacks of limited specificity and insufficient delivery commonly observed with antisense oligonucleotides (27). Elucidation of the mechanisms regulating Survivin and EPR-1 gene expression should further facilitate the selective disruption of this novel anti-apoptosis pathway in cancer without affecting viability of normal tissues.

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