ANTICANCER RESEARCH *24*: 425-432 (2004)

Construction and Transfection of a Ribozyme Targeting Human Caspase-3

DEIRDRE CRONIN[1], CARMEL DALY[1], TONI O'DOHERTY[2], MARY HEENAN[1], KEVIN J. SCANLON[3] and MARTIN CLYNES[1]

[1]*National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9;*
[2]*Archport, Dublin City University, Glasnevin, Dublin 9, Ireland;*
[3]*Keck Graduate Institute, 535 Watson Drive, Claremont, CA 91711, U.S.A.*

Abstract. *Caspase-3 is a key executioner cysteine protease involved in programmed cell death or apoptosis. A ribozyme to human caspase-3 was designed, tested by in vitro cleavage, and transfected into a drug-resistant variant (DLKP-A5F) of a human lung carcinoma cell line (DLKP). By both stable and transient transfection, this ribozyme was shown to be effective at down-regulating human caspase-3 mRNA and protein levels.*

Many cell death stimuli, including growth factor withdrawal, receptor ligation, drug treatment and virus infection, have been shown to activate the cellular family of cysteine proteases known as caspases (1-4). This protein family exists in cells as inactive precursors. Upon activation by a death stimulus, caspases are cleaved into two subunits, usually 20kDa and 10kDa, at aspartate residues (5, 1, 6). These domains then form a heterotetramer, the active enzyme. Once activated, these proteins initiate and execute a number of apoptotic pathways in cells (7, 1, 8, 2).

All caspases contain the amino acid sequence QACxG (tryptophan-aspartate-cysteine-x-glutamate) which contains the active cysteine. The protease activity of the caspase family is unique in that they cleave following aspartate residues (asp x). Active caspases can activate other caspases following an initial activating stimulus as well as cleaving themselves (5, 6).

Caspase-3 (CPP-32) is a key executioner protein. It is a 32kDa protein widely distributed in many tissues. Purified caspase-3 can cleave PARP, DFF (DNA fragmentation factor), fodrin, sterol-regulatory element-binding proteins (SREBPs), U1-associated 70kDa protein, huntingtin, DNA-dependent protein kinase, *etc* (1, 9). Differences in levels of CPP-32 in cells may influence the relative sensitivity or resistance to apoptosis. Caspase-3 activation appears to be a downstream consequence of anticancer drug treatment and some studies document a down-regulation of caspase-3 in multi-drug-resistant cancer cells (10, 11).

Ribozymes are non-protein enzymes that catalyse RNA-cleavage in a sequence-specific way (12, 13, 14). They can be synthesised as tools to inhibit the expression of specific RNA transcripts and, as they have the ability to cleave deleterious RNAs or repair mutant cellular RNAs, have potential therapeutic benefits. Their structures are based on naturally occurring site-specific, self-cleaving RNA molecules (12). Hammer-head ribozymes cleave their target RNA directly after NUX sites, where X= C, U or A, and N= any nucleotide. The GUC triplet is the preferred site (15, 16). The sequences of the adjacent stems determine the specificity of these molecules for their target (17, 18). Cleavage depends on the presence of divalent metal ions at neutral or higher pH and results in the production of two truncated RNA molecules (19).

Previous studies have used hammerhead ribozymes to target rat caspase-3 in neuronal cells (20, 21) and antisense oligonucleotides against human caspase-3 have been described (by Los and co-workers) (22). Here, we describe the design of the first reported ribozyme targeting human caspase-3.

Materials and Methods

Cell lines. DLKP is a poorly-differentiated human squamous cell line, previously described (23, 24). DLKP-A5F is a clonal cell population derived from the adriamycin-selected variant, DLKP-A and this clone is 300-fold resistant to doxorubicin and overexpresses the mdr-1 drug efflux pump, P-glycoprotein (25).

Chemicals and antibodies. Adriamycin was obtained from Farmitalia Carlo Erba Ltd., U.K. All media used in the maintenance of the cell lines was obtained from Gibco BRL, Life Technologies, U.K. Anti-caspase-3 antibodies were purchased from

Correspondence to: Martin Clynes, National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland.

Key Words: Ribozyme, caspase-3.

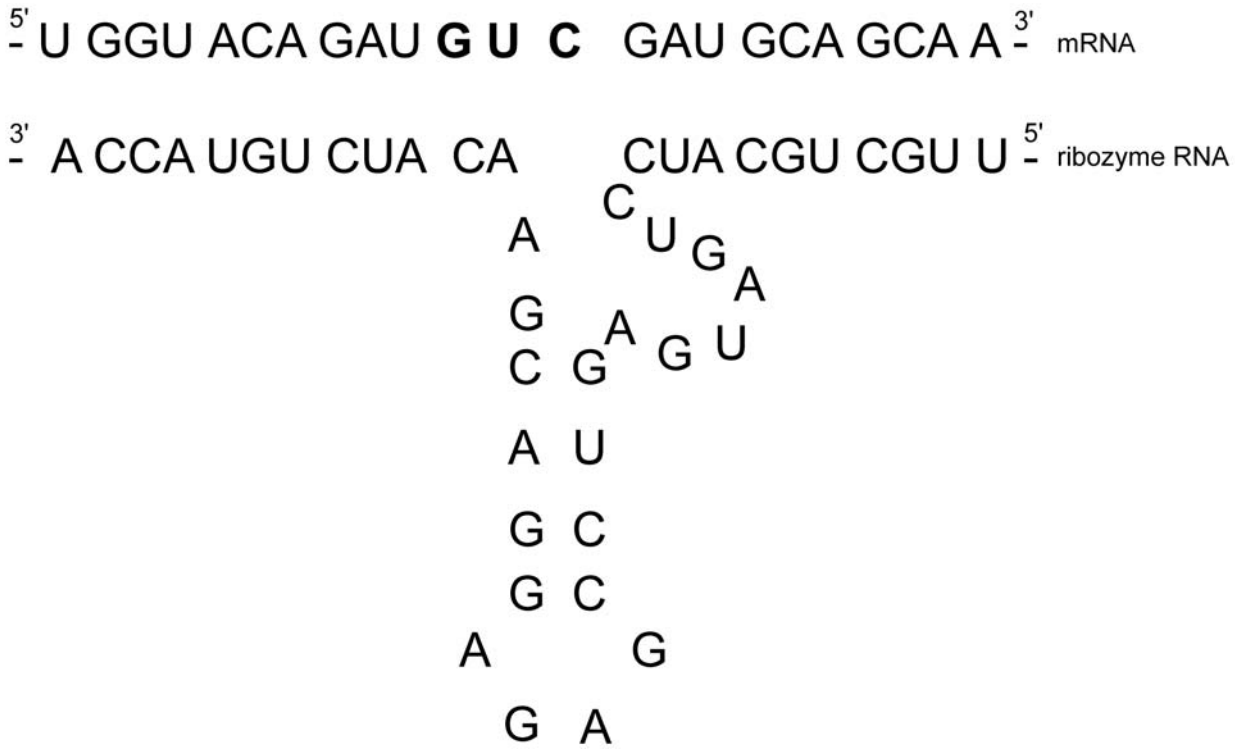

Figure 1. *Structure and sequence of the caspase-3 hammerhead ribozyme (Rz1).*

Transduction laboratories (Affiniti, U.K.). All other chemicals were obtained from Sigma Chemical Co., U.K.

Selection of ribozyme target site. M-fold is a computer program that enables RNA secondary structure prediction. Developed by Michael Zuker and colleagues, it allows the prediction of the minimum free energy or optimal secondary structures of target RNAs (26, 27). The specificity of the ribozyme was determined using BLAST (NCBI homepage).

In vitro cleavage using $MgCl_2$ gradient. RNA transcripts of caspase-3 cDNA and ribozyme 1 were generated using T7 Riboprobe System (Promega, P1440) and purified using phenol-chloroform gradients followed by final purification using Quick Spin column (Boehringer Mannheim, 1273990) according to manufacturer's protocols. Each 10μl reaction contained 50mM Tris-HCl, pH8.0, 40U RNasin, 0.1μg of human caspase-3 RNA, 1μg of ribozyme (or water for control reactions) and $MgCl_2$. The magnesium concentrations used were 0mM, 5mM, 10mM and 15mM. Mixtures were incubated at 37˚C for 24 hours and stopped by the addition of 2X loading dye. Reaction products were heat-denatured at 90˚C for 3 minutes and then separated on 12% polyacrylamide gels containing 7M urea (Sigma, U-5378).

Ribozyme preparation. Ribozyme 1 (Rz1) and Ribozyme 1 Reverse (Rz1R) (see details in Results Section) were cloned into Promega's pTARGET vector, a CMV promoter-based expression vector containing the gene for neomycin selection. Primers used to construct Rz1 are:

Primer 1: TTG CTG CAT CCT GAT GAG TCC CGT GAG GAC GAA ACA TCT GTA CCA
Primer 2: TGG TAC AGA TGT TTC GTC CTC ACG GGA CTC ATC AGG ATG CAG CAA

The full-length coding region of caspase-3 (used for *in vitro* cleavage experiments), which was cloned into Invitrogen's pcDNA3, a CMV promoter-based expression vector containing the gene for neomycin selection, was a generous gift from Prof. V. Dixit and is described in 28. The β-galactosidase gene was contained in the pCH110 plasmid (Invitrogen) and was used to estimate transfection efficiency.

Transient transfection of ribozyme and reverse ribozyme into DLKP-A5F cells. To demonstrate the ability of the ribozyme to cleave human caspase-3, DLKP-A5F cells were transiently transfected with Rz1 or Rz1R using Fugene-6, (Roche) (method used as described by Roche).

RNA analysis. Gene transcript levels of caspase-3 and β-actin (acting as a housekeeper gene) were analysed by semi-quantitative RT-PCR method. The transcripts were analysed within their exponential range and β-actin, used as an internal control, was co-amplified with the gene of interest. An annealing temperature of 54˚C was used in all amplifications and the resulting PCR products were analysed by agarose gel electrophoresis and their size determined by comparison with the ΦX174 DNA Hae III digested molecular weight marker (Sigma). The primer sequence and product size for each gene are as follows. Caspase-3: (forward) 5'GAA TGA CAT CTC GGT CTG3'; (reverse) 5'ACG GCA GGC

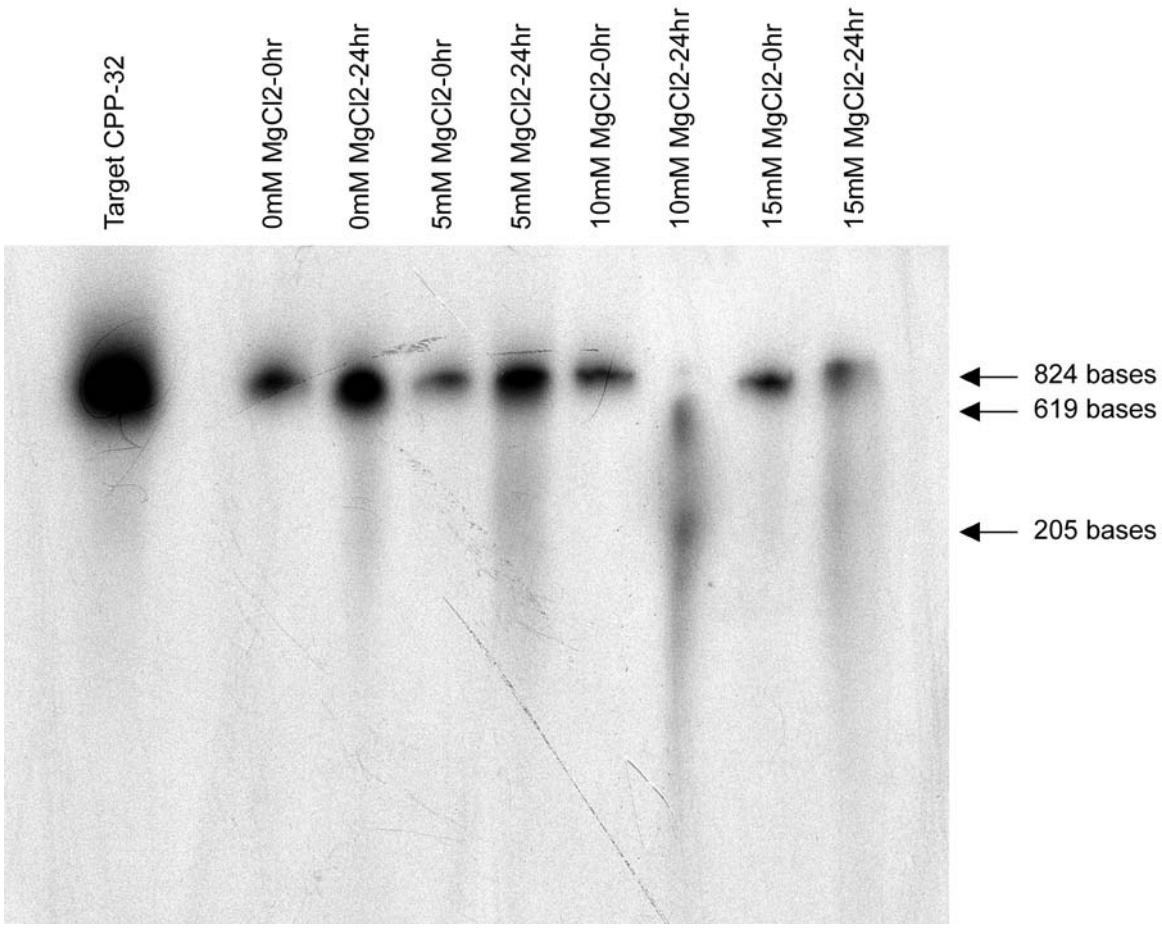

Figure 2. *IVC optimisation using magnesium gradient (15% acrylamide gel). Lane 1 contains target caspase-3 and no ribozyme. Each lane following contains increasing concentrations of $MgCl_2$ at 0h and 24h. Cleavage products are indicated by arrows at position 619 and 205 base pairs. Full size product is indicated at 824 bases.*

CTG AAT AAT3'; PCR cycle number, 30; product length (bp), 314. B-actin: (forward) 5'TGG ACA TCC GCA AAG ACC TGT AC3'; (reverse) 5'TCA GGA GGA GCA ATG ATC TTG A3'; product length (bp), 142.

Protein analysis. Protein expression was determined by Western blot analysis using standard methods.

Results

Design and construction of the caspase-3 ribozyme. A caspase-3 ribozyme has been constructed targeting rat caspase-3 (20). However, when human and rat caspase-3 mRNA sequences were aligned, no corresponding target site was found in the human mRNA. Human caspase-3 mRNA was analysed for its GUC sites (Clustal W & multiple sequence alignment computer package). There are a total of six GUC sites in the caspase-3 mRNA.

As well as examining the optimal secondary structure of caspase-3 mRNA, another nine suboptimal mRNA folding patterns were studied. When the GUC sites were analysed using m-fold structure dot plot analysis for mRNA of human caspase-3 (22), it was found that single stranded loop regions were common to all predicted structures around nucleotides 205, 250 and 750.

A hammerhead ribozyme was constructed to target caspase-3 at position 205, which is within the p17 subunit of the protein (see Materials and Methods for sequence details). The specificity of the ribozyme for caspase-3 was determined by BLAST sequence analysis of all human sequences currently in the genbank. There was no homology to any other human sequence, so theoretically the ribozyme was specific for caspase-3 alone. The ribozyme sequence was cloned into the pTARGET vector (Promega). In addition to ribozyme 1, a non-functioning sequence was cloned into

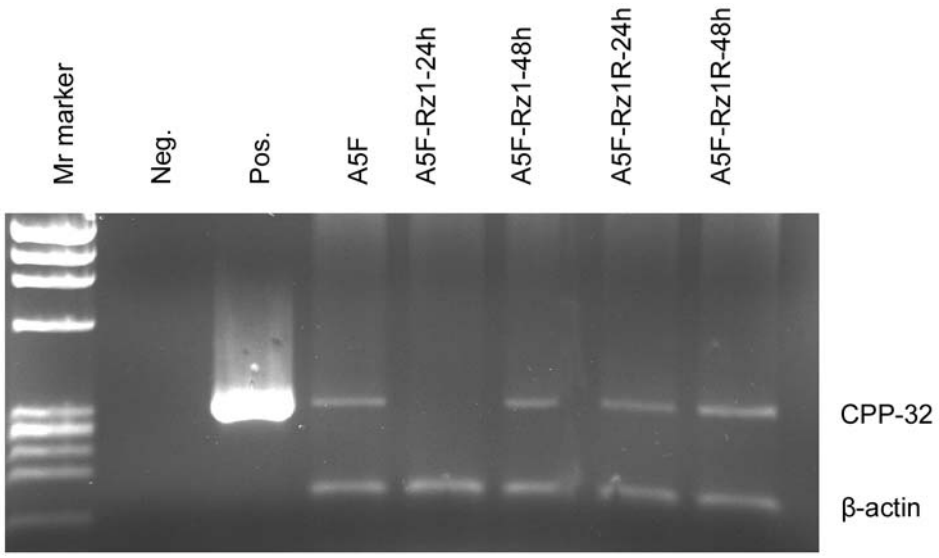

Figure 3. *RT-PCR analysis of CPP-32 expression in transiently transfected cells. The molecular weight marker (Mr marker) is "φ-X174" Hae III digest (Promega: G1761). Caspase-3 primers amplify a product of 314 bp. Internal control β-actin amplified to yield a 142bp product. Gel shows caspase-3 levels in control, ribozyme 1 and reverse ribozyme transfectants.*

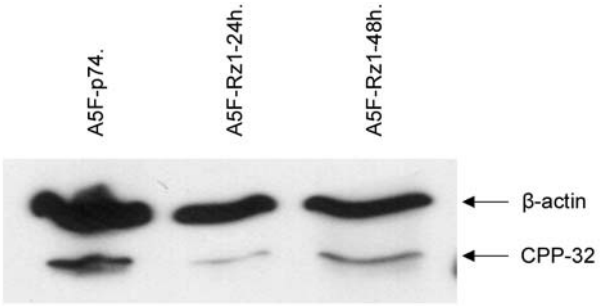

Figure 4. *Western blot analysis displaying pro-caspase-3 levels in control and transfected cells. Pro-caspase-3 is a 32kDa protein. β-actin was used as a control and is a 42 kDa protein. Protein was separated on a 12% SDS-polyacrylamide gel. Twenty-five ug of protein was loaded in each case.*

the pTARGET vector (see Materials and Methods for sequence details). This reversed sequence was complementary to that of the ribozyme and therefore identical to that of the caspase-3 mRNA. This Rz1R acted as a control plasmid, unable to cleave the caspase-3 mRNA sequence. Figure 1 shows the hammerhead structure of ribozyme 1.

In vitro cleavage (IVC) analysis of the caspase-3 ribozyme. The ability of the ribozyme to target caspase-3 was initially determined by assessing its ability to cleave caspase-3 mRNA *in vitro*. Ribozyme (42 bases) and target sequences (824 bases) had been cloned into pTARGET (Promega) and pcDNA3.1 (Invitrogen) vectors, respectively. The T7 polymerase sequence in both vectors facilitates their use in *in vitro* transcription reactions to produce radiolabelled caspase-3 ribozyme and caspase-3 target. The radiolabelled ribozyme and target RNA were then combined in the *in vitro* cleavage reaction and the products were separated on a polyacrylamide gel.

$MgCl_2$ initiates the cleavage reaction (29, 30). To optimise the IVC reaction, it was necessary to use a $MgCl_2$ gradient. The absolute requirement of the IVC reaction for divalent metal ions is demonstrated by Figure 2. The caspase-3 transcript is unaltered by incubation with caspase-3 Rz1 in the absence of $MgCl_2$. The IVC cleavage of caspase-3 target mRNA proceeds at an optimal concentration of 10mM $MgCl_2$ and at a temperature of 37˚C. Time course analysis shows that after 24 hours, substrate concentration decreases and cleavage products appear (205 and 619bps). It is thought that a certain amount of ribozyme activity will take place at suboptimal conditions and this is evident in Figure 2 which illustrates that at each magnesium concentration after 24 hours a "bleed" of signal appears on the X-ray. This is particularly evident at 5mM and 15 mM $MgCl_2$. The amount of the CPP-32 target sequence decreases after 24hours, while those of the cleavage bands appear after 24hours. This is entirely consistent with a time-dependent cleavage of the CPP-32 target sequence by the CPP-32 ribozyme.

RT-PCR for CPP-32 expression in transiently transfected A5F cells. The effectiveness of the caspase-3 ribozyme *in vivo* was assessed using a transient transfection assay. Using a β-galactosidase assay, a transfection efficiency of 35% was achieved in DLKP-A5F cells. DLKP-A5F cells were transiently transfected with ribozyme (Rz1) and control reverse ribozyme (Rz1R) to caspase-3 using the optimised transfection protocol. To determine if the cells displayed a reduction in caspase-3 mRNA levels compared to parent or reverse ribozyme transfectants, RT-PCR was carried out on total RNA isolated from each transfectant. Cells were harvested for RNA extraction at 24 and 48 hours after transfection. The expression of caspase-3 mRNA was decreased to very low levels at 24 hours (see Figure 3). At 48 hours, caspase-3 expression had increased but was still not back to the levels of the nontransfected or reverse ribozyme transfectants.

Western blot analysis of pro-caspase-3 levels in transiently transfected A5F cells. Western blot analysis showed a decrease in pro-caspase-3 protein levels at 24 h and to a lesser extent at 48 h post transient transfection. β-actin was used as an internal control. Results are presented in Figure 4.

Discussion

This is the first reported ribozyme targeting human caspase-3. Previous studies (20, 21) have used ribozymes to rat caspase-3 in the context of neurological studies. Eldadah and coworkers found that ribozyme expression conferred protection against apoptosis at 24hours post-transfection (20). Xu and coworkers found that ribozyme-transfected PC12 cells had increased protection against apoptosis induced by low levels of 6-hydroxydopamine when compared with non-transfected cells (21).

When choosing a ribozyme target site, it is desirable to select a target NUX site in an area of single-stranded RNA which allows target to base pair with its complementary sequence on the ribozyme, and also ensures that the catalytic moiety of the ribozyme, (NUX site), can access the target region (31). Given the complexities of RNA folding patterns, we used M-Fold, a programme that predicts the most stable mRNA folding pattern based on calculating the minimum free energy (26, 27). Analysis of the six GUC sites in the caspase-3 mRNA using structure dot plot analysis for mRNA of human caspase-3 revealed that loops occur in all predicted structures around nucleotides 200, 250 and 750. Ribozyme 1 was chosen to target position 205 of human caspase-3, which lies within the p17 subunit of the protein.

Hammerhead ribozymes depend on divalent cations for binding and cleavage of their substrate (32, 33, 29, 30). An *in vitro* cleavage assay demonstrated that the purified ribozyme was capable of cleaving the target sequence *in vitro*.

The magnesium gradient in Figure 2 illustrates how this ribozyme's activity *in vitro* is affected by magnesium concentration. Each cleavage reaction at different magnesium concentrations showed a "bleed" of signal after 24hours incubation. This was probably due to ribozyme activity taking place at suboptimal conditions. *In vitro* cleavage of CPP-32 target cDNA proceeded at an optimal concentration of 10mM $MgCl_2$ – consistent with a previous report (34).

The effectiveness of this caspase-3 ribozyme in living cells was illustrated by developing a transient transfection assay. The use of transient transfection, particularly in apoptosis research, has been widely reported (22, 35-39). It is a useful tool to illustrate the short-term effects of induced gene expression and has become a fast and effective method in our lab for investigating ribozyme function.

To achieve high expression efficiency, most studies use viral vectors thereby obtaining very high viral replication and hence induced gene expression (39-42). However in this study, non-viral mammalian expression vectors were transfected into DLKP-A5F cells with 35% transfection efficiency. This level of transfection efficiency enabled sufficient ribozyme activity resulting in decreases in caspase-3 at both the mRNA and protein levels in transiently transfected cells. Other studies where cDNA and ribozyme to rat caspase-3 were investigated used transient transfection to illustrate the ribozyme's efficacy (20, 35, 21).

DLKP-A5F are a clonal population which display 300-fold resistance to adriamycin and low levels of caspase-3 when compared with their drug sensitive parental population, DLKP (25). Given these low levels, any change in caspase-3 expression due to ribozyme activity should be easily determined. Our results indicate that the caspase-3 ribozyme transfection resulted in a decrease in the expression of caspase-3 mRNA and protein. A decrease in mRNA was evident at 24hours and in protein at 24 and 48 hours.

This study involved the design and construction of the first ribozyme to human caspase-3. It is also the first study where a ribozyme to caspase-3 was used in cancer cells. This ribozyme functions both *in vitro* and *in vivo*, as illustrated by *in vitro* cleavage, RT-PCR and Western blot. The ribozyme proved effective at decreasing RNA and protein with some effects on drug resistance levels (data not shown).

There are many studies that document a down-regulation of caspase-3 in Pgp overexpressing cells and more aggressive cancers (11, 10). Drug resistant cervical cancer cells had reduced levels of caspase-3 (43). Procaspase-3 expression has been correlated with decreased incidence of lymph node metastases and longer median survival in patients with non-small cell lung cancer (44). Ruefli and coworkers found that Pgp+ cells were less sensitive to those nuclear apoptotic events that occur following caspase activation (10).

The caspase-3 ribozyme described here, in association with the mdr-1 ribozyme previously described (45), may be useful for further investigation of the interactions between P-glycoprotein and caspase-3.

References

1 Kohler C, Orrenius S and Zhivotovsk B: Evaluation of caspase activity in apoptotic cells. J Immunological Methods *265*: 97-100, 2002.

2 Slee E and Martin S: Regulation of caspase activation in apoptosis: implications for transformation and drug resistance. *In*: Multiple Drug Resistance in Cancer 2. Molecular, Cellular and Cancer Aspects. Ed. Martin Glynes. Kluwer Academic Publishers pp 309-321, 1998.

3 Zheng T, Huno S, Kuida K, Momoi T, Srinivasan A, Nicholson D, Lazebnik Y and Flavell R: Deficiency in caspase-9 or caspase-3 induces compensatory caspase activation. Nature Medicine *6(11)*: 1241-7, 2000.

4 Weber C and Vincenz C: A docking model of key components of the DISC complex: death domain superfamily interactions redefined. FEBS Letters *492*: 171-176, 2001.

5 Fadeel B, Orrenius S and Zhivotovsky B: The most unkindest cut of all: on the multiple roles of mammalian caspases. Leukemia *14(8)*: 1514-25, 2000.

6 Song Z and Steller H: Death by design: mechanism and control of apoptosis. Trends Cell Biol *9(12)*: M49-52, 1999.

7 Bowen C, Voeller H, Kiklv K and Gelmann E: Synthesis of procaspases-3 and -7 during apoptosis in prostate cancer cells. Cell Death Differ *6(5)*: 394-401, 1999.

8 Waterhouse N and Trapani J: CTL : Caspases terminate life, but that's not the whole story. Tissue Antigens *59(3)*: 175-83, 2002.

9 Liu Q and Hengartner M: The molecular mechanism of programmed cell death in *C. elegans*. Ann NY Acad Sci *887*: 92-104, 1999.

10 Ruefli A, Bernhard D, Tainton K, Kofler R, Smyth M and Johnstone R: Suberoylanilide hydroxamic acid (SAHA) overcomes multidrug resistance and induces cell death in P-glycoprotein-expressing cells. Int J Cancer *99(2)*: 292-8, 2002.

11 Johnstone R, Cretney E and Smyth M: P-glycoprotein protects leukaemia cells against caspase-dependent, but not caspase-independent cell death. Blood *93(2)*: 1075-1085, 1999.

12 Cech T, Zaug A and Grabowski P: *In vitro* splicing of the ribosomal RNA precursor of Tetrahymena: involvement of a guanosine nucleotide in the excision of the intervening sequence. Cell *27*: 487-496, 1981.

13 Cech T: Ribozymes, the first 20 years. Biochem Soc Trans *30(6)*: 1162-6, 2001.

14 Freelove A and Zheng R: The power of ribozyme technologies: the logical way ahead for molecular medicine and gene therapy? Curr Opin Mol Therap *4(5)*: 419-22, 2002.

15 Palfner K, Kneb, M, Hiddemann W and Bertram J: Improvement of hammerhead ribozymes cleaving mdr-1 mRNA. Biol Chem Hoppe-Seyler *376*: 289-295, 1995.

16 Esteban J, Walter N, Kotzorek G, Heckman J and Burke J: Structural basis for heterogeneous kinetics: Reengineering the hairpin ribozyme. Proc Natl Acad Sci USA *95*: 6091-6096, 1998.

17 Takagi Y, Suyama E, Kawasaki H, Miyagishi M and Taira K: Mechanism of action of hammerhead ribozymes and their applications *in vivo*: rapid identification of functional genes in the post-genome era by novel hybrid ribozyme libraries. Biochemical Soc Trans *30(6)*:1145-9, 2001.

18 Castanotto D, Li J, Michienzi A, Langlois M, Lee N, Puymirat J and Rossi J: Intracellular ribozyme applications. Biochemical Soc Trans *30(6)*: 1140-5, 2001.

19 Doudna J and Cech T : The chemical repertoire of natural ribozymes. Nature *418 (6894)*: 222-8, 2002.

20 Eldadah B, Ren R and Faden A: Ribozyme-mediated inhibition of caspase-3 protects cerebellar granule cells from apoptosis induced by serum-potassium deprivation. J Neuroscience *20 (1)*: 179-186, 2000.

21 Xu RH, Lie J, Chen XW, Xu F, Xie Q, Yu H, Guo Q, Zhou XQ and Jin YX: Ribozyme-mediated inhibition of caspase-3 activity reduces apoptosis induced by 6-hydroxydopamine in PC12 cells. Brain Res *899*: 10-19, 2001.

22 Los M, Herr I, Friesen C, Fulda S, Schulze-Osthoff K and Debatin KM: Cross-resistance of CD95- and drug-induced apoptosis is a consequence of deficient activation of caspases (ICE/Ced-3 proteases). Blood *90(8)*: 3118-3129, 1997.

23 Law E, Gilvarry U, Grant G, Gregory B and Clynes M: Cytogenetic comparison of 2 poorly differentiated human lung squamous cell carcinoma cell lines. Cancer Gen Cytogenet *59*: 111-118, 1992.

24 McBride S, Meleady P, Baird A, Dinsdale D and Clynes M: Human lung carcinoma cell line DLKP contains 3 distinct subpopulations with different growth and attachment properties. Tumor Biology *19(2)*: 88-103, 1998.

25 Heenan M, O'Driscoll L, Cleary I, Connolly L and Clynes M: Isolation from a human MDR lung cell line of multiple clonal populations which exhibit significantly different drug resistance. Int J Cancer *71(5)*: 907-15, 1997.

26 Zuker M, Jaeger JA and Turner DH: A comparison of optimal and suboptimal RNA secondary structures predicted by free energy minimization with structures determined by phylogenetic comparison. Nucleic Acids Res *19(10)*: 2707-14, 1991.

27 Zuker M: http://mfold2.wustl.edu RNA mfold program.

28 Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS and Dixit VM: Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. Cell *81(5)*: 801-9, 1995.

29 Dorai T, Olsson C, Katz A and Buttyan R: Development of a hammerhead ribozyme against bcl-2. 1. Preliminary evaluation of a potential gene therapeutic agent for hormone-refractory human prostate cancer. The Prostate *32*: 246-258, 1997.

30 Chang P, Cantin E, Zaia J, Ladne P, Stephens D, Sarver N and Rossi J: Ribozyme-mediated site-specific cleavage of the HIV-1 genome. Clinical Biotechnology *2*: 1, 1990.

31 Amarzguioui M, Brede G, Babaie E, Grotli M, Sproat B and Prydz H: Secondary structure prediction and *in vitro* accessibility of mRNA as tools in the selection of target sites for ribozymes. Nucleic Acids Res *28(21)*: 4113-4124, 2000.

32 Hormes R and Sczakiel G: The size of hammerhead ribozymes is related to cleavage kinetics : the role of substrate length. Biochimie *84(9)*: 897-903, 2002.

33 Tzokov S, Murray I and Grasby J: The role of magnesium ions and 2'hydroxyl groups in the VS ribozyme-substrate interaction. J Mol Biol *324(2)*: 215-26, 2002.

34 James HA and Gibson I: The Therapeutic Potential of Ribozymes. Blood *91(2)*: 371-382, 1998.
35 Wrone-Smith T, Izban K, Ergin M, Cosar E, His E and Alkan S: Transfection of caspase-3 in the caspase-3-deficient Hodgkin's disease cell line, KMH2, results in enhanced sensitivity to CD95-, TRAIL-, and ARA-C-induced apoptosis. Exp Hematol *29*: 572-581, 2001.
36 Katoh O, Takahashi T, Oguri T, Kuramoto K, Mihara K, Kobayashi M, Hirata S and Watanabe H: VEGF inhibits apoptosis death in hematopoietic cells after exposure to chemotherapeutic drugs by inducing Mcl-1 acting as an anti-apoptotic factor. Cancer Res *58*: 5565-5569, 1998.
37 Townsend AJ, Fields WR, Doss AJ, Clapper ML, Doehmer J and Morrow CS: Modeling the chemoprotective functions of glutathione S-transferases in cultured cell lines by heterologous expression. Drug Metab Rev *31(1)*: 43-69, 1999.
38 Kuo ML, Chuang SE, Lin MT and Yang SY: The involvement of PI 3-K/Akt-dependent up-regulation of mcl-1 in the prevention of apoptosis of Hel3B cells by interleukin-6. Oncogene *20*: 677-685, 2001.
39 Friedrich K, Wieder T, Von Haefen C, Radetzki S, Janicke R, Schulze-Osthoff K, Dorken B and Daniel PT: Over-expression of caspase-3 restores sensitivity for drug-induced apoptosis in breast cancer cell lines with acquired drug resistance. Oncogene *20(22)*: 2749-60, 2001.
40 Lotze M and Kost T: Viruses as gene delivery vectors: Application to gene function, target validation, and assay development. Cancer Gene Therapy *9*: 692-699, 2002.
41 Cemazar M, Sersa G, Wilson J, Tozer G, Hart S, Grosel A and Dachs G: Effective gene transfer to solid tumours using different nonviral gene delivery techniques: Electroporation, liposomes, and integrin-targeted vector. Cancer Gene Therapy *9*: 399-416, 2002.
42 Mendoza-Maldonado R, Zentilin L, Fanin R and Giacca M: Purging of chronic myelogenous leukaemia cells by retrovirally expressed anti-bcr-abl ribozymes with specific cellular compartmentalization. Cancer Gene Therapy *9*: 71-86,2002.
43 Ding Z, Yang X, Pater A and Tang S: Resistance to apoptosis is correlated with the reduced caspase-3 activation and enhanced expression of antiapoptotic proteins in human cervical multidrug-resistant cells. Biochem Biophys Res Commun *270(2)*: 415-20, 2000.
44 Mow B, Blajeski AL, Chandra J and Kaufmann SH: Apoptosis and the response to anticancer therapy. Curr Opin Oncol. *13(6)*: 453-62, 2001.
45 Daly C, Coyle S, McBride S, O'Driscoll L, Daly N, Scanlon K and Clynes M: MDR-1 ribozyme mediated reversal of the multi-drug resistant phenotype in human lung cell lines. Cytotechnology *19*: 199-205, 1996.

Received September 17, 2003
Accepted January 5, 2004