Cell Death and Differentiation (2006) 13, 346–347
© 2006 Nature Publishing Group All rights reserved 1350-9047/06 $30.00
www.nature.com/cdd

Letter to the Editor

Evaluation of the role of caspase-6 in anticancer drug-induced apoptosis

Cell Death and Differentiation (2006) **13,** 346–347. doi:10.1038/sj.cdd.4401791; published online 18 November 2005

Dear Editor,

According to current understanding, caspase-6 is an effector caspase that is activated downstream of caspase 3 during apoptosis[1,2] and is essential only for the apoptotic cleavage of lamin A in nuclei that express this polypeptide.[3] On the other hand, several recent observations have raised the possibility that caspase 6 might also play an important upstream role during anticancer drug-induced apoptosis. First, based on the ability of IETD-fmk but not crmA or caspase 8 deficiency to inhibit resveratrol-induced apoptosis in human T-cell leukemia cell lines, it has been suggested that caspase 6 is the initiator caspase after treatment with this experimental anticancer agent.[4] Second, DU145 human prostate cancer cells selected for camptothecin resistance were observed to have diminished expression of procaspase-6.[5] More recently, experiments designed to measure procaspase polypeptide levels[6] in the 60 human tumor cell lines utilized by the National Cancer Institute to screen potential anticancer drugs[7,8] demonstrated a striking correlation between procaspase 6 polypeptide levels and ability of certain agents to induce cell death (LC_{50}) across the cell lines (Figure 1a and molecular target MT1646 at http://www.dtp.nci.nih.gov/webdata.html). Collectively, these observations raised the possibility that caspase 6 might play a previously unsuspected critical role in the induction of apoptosis after treatment with selected anticancer drugs.

To test this hypothesis, we examined the ability of a number of agents to induce apoptosis in parental and *caspase-6*$^{-/-}$ DT40 chicken lymphoma cells.[3] Our data revealed that the ability of the topoisomerase II poison etoposide to induce apoptosis in the *caspase-6*$^{-/-}$ cell line was intact (Figure 1b) despite the absence of detectable procaspase-6 protein (Figure 1c), ruling out the possibility that the gene targeting and selection process had raised the apoptotic threshold in this cell line. When treated with camptothecin, the parental and caspase-6$^{-/-}$ cells displayed roughly equal sensitivity to the induction of apoptosis as assessed by the appearance of cells with subdiploid DNA content after propidium iodide staining (Figure 1d) or the activation of caspase 3-like activity (Figure 1e).

Among the compounds that showed a high correlation between procaspase-6 expression and LC_{50}, several were not available for testing (not shown) or failed to induce apoptosis in DT40 cells after 48 h of continuous exposure to the highest tested concentration (Figure 1a). Three of the agents, however, readily induced apoptosis in DT40 cells (Figure 1a and f). Of these three, two (NSC 349856 and NSC 682298) induced slightly more apoptosis in *caspase-6*$^{-/-}$ DT40 cells than parental cells (Figure 1f). In short, the loss of caspase-6 does not appear to impair the ability of any of these agents to induce apoptosis. Even though the correlations between caspase-6 content and drug sensitivity across 60 cell lines were striking and highly statistically significant for several compounds (Figure 1a), we found no mechanistic basis for these correlations and no evidence that caspase 6 plays an essential role in the cytotoxic response to these agents.

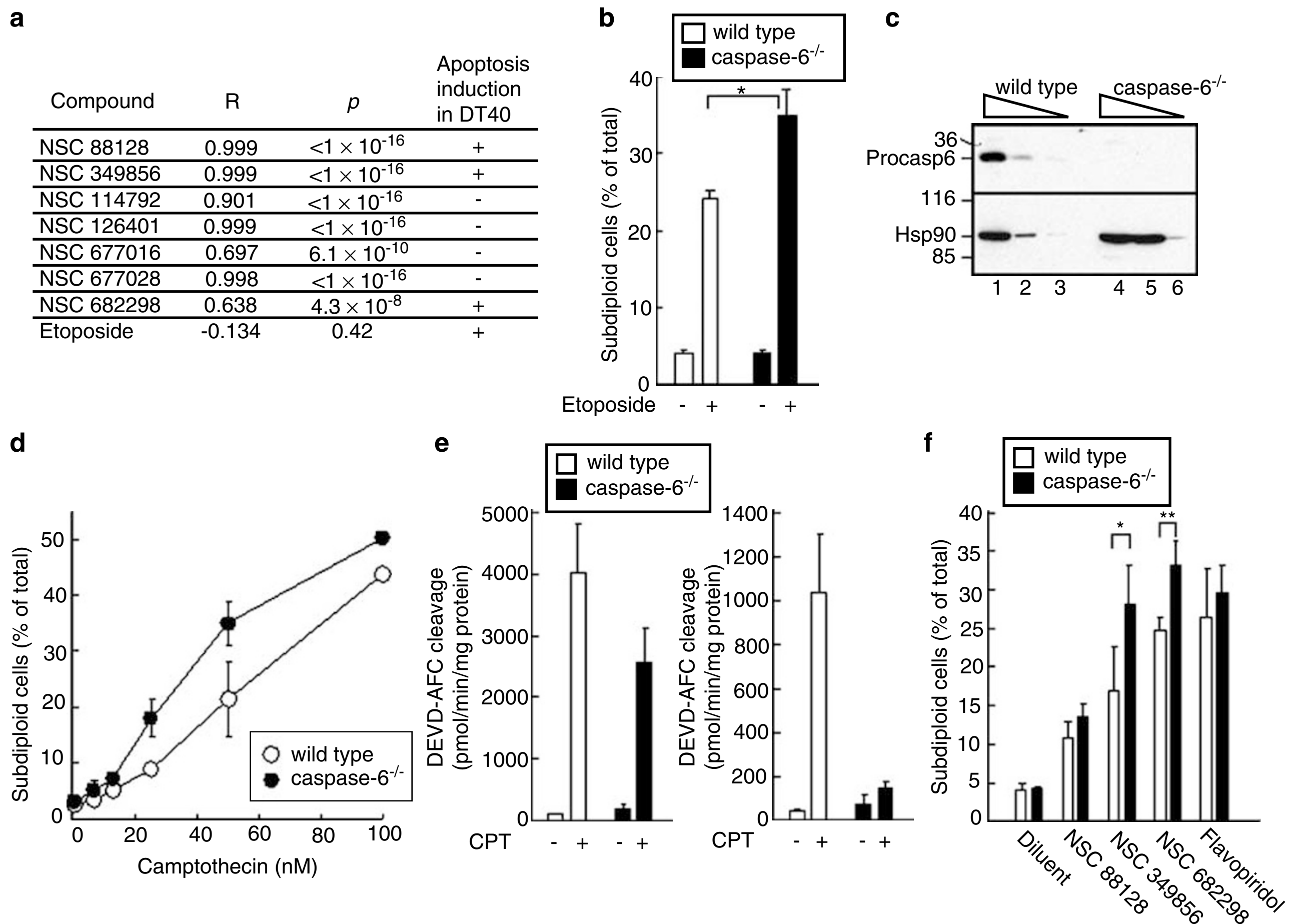
Acknowledgements

We thank Dominic Scudiero, Edward Sausville and Susan Holbeck for their assistance in identifying the correlation between procase-6 expression and sensitivity of the 60 cell lines to various agents. Work in our laboratories is supported by Grant CA69008 from the National Cancer Institute.

DA Loegering[1], S Ruchaud[2], WC Earnshaw[2] and SH Kaufmann[*,1,3]

[1] Division of Oncology Research, Mayo Clinic College of Medicine, Rochester, MN 55905 USA
[2] Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland, UK
[3] Department of Molecular Pharmacology, Mayo Clinic College of Medicine, Rochester, MN 55905 USA
[*] Corresponding author: S Kaufmann, Division of Oncology Research, Guggenheim 1342 C, Mayo Clinic, 200 First St, S.W., Rochester, MN 55905, USA. Tel: +1 507 284 8950; Fax: +1 507 284 3906; E-mail: Kaufmann.scott@mayo.edu

1. Srinivasula SM *et al.* (1998) Mol. Cell. 1: 949–957
2. Slee EA *et al.* (1999) J. Cell Biol. 144: 281–292
3. Ruchaud S *et al.* (2002) EMBO J. 21: 1967–1977
4. Bernhard D *et al.* (2000) Cell Death Differ. 7: 834–842
5. Reinhold WC *et al.* (2003) Cancer Res. 63: 1000–1011
6. Svingen PA *et al.* (2004) Clin. Cancer Res. 10: 6807–6820
7. Boyd MR and Paull KD (1995) Drug Dev. Res. 34: 91–109
8. O'Connor PM *et al.* (1997) Cancer Res. 57: 4285–4300
9. Nicoletti I *et al.* (1991) J. Immunol. Methods 139: 271–279
10. Mesa RA *et al.* (2005) Blood 106: 318–327
11. Kaufmann SH (2001) Anal. Biochem. 296: 283–286
12. Martins LM *et al.* (1997) J. Biol. Chem. 272: 7421–7430
13. Chao SH and Price DH (2001) J. Biol. Chem. 276: 31793–31799
14. Decker RH, Dai Y and Grant S (2001) Cell Death Differ. 8: 715–724



Compound	R	p	Apoptosis induction in DT40
NSC 88128	0.999	$<1 \times 10^{-16}$	+
NSC 349856	0.999	$<1 \times 10^{-16}$	+
NSC 114792	0.901	$<1 \times 10^{-16}$	-
NSC 126401	0.999	$<1 \times 10^{-16}$	-
NSC 677016	0.697	6.1×10^{-10}	-
NSC 677028	0.998	$<1 \times 10^{-16}$	-
NSC 682298	0.638	4.3×10^{-8}	+
Etoposide	-0.134	0.42	+

Figure 1 Relationship between procaspase-6 expression and drug sensitivity. (**a**) Correlation between procaspase 6 content and sensitivity to certain drugs. For each compound, R represents the Pearson correlation coefficient for results of assays that independently determined the relative procaspase-6 polypeptide levels in 60 human cancer cell lines by immunoblotting as recently described[6] and the drug concentration that decreased cell mass (determined by sulforhodamine blue binding after a 48 h exposure)[7,8] to 50% of pretreatment levels (LD_{50}) in the same cell lines. A positive *R* value indicates that cells with high procaspase 6 levels are more sensitive. *p* represents the probability that this correlation would arise by chance alone. Structures of the compounds can be found at http://www.dtp.nci.nih.gov/webdata.html. The ability of these agents to induce apoptosis in DT40 cells as assessed by flow cytometry after treatment at 5 μg/ml for 48 h followed by propidium iodide staining and flow cytometry (see below) is also indicated. + indicates apoptosis, −indicates no apoptosis. (**b**) Comparison of etoposide sensitivity of parental and *procaspase-6*$^{-/-}$ DT40 cells. After a 24 h exposure to 1 μM etoposide, cells were stained with 50 μg/ml propidium iodide in 0.1% (w/v) sodium citrate containing 0.1% (w/v) Triton X-100 at 4°C overnight and then subjected to flow microfluorimetry as previously described.[9,10] In this panel and subsequent panels, parental cells and caspase-6$^{-/-}$ cells are represented by open and closed symbols, respectively. Bars, mean ± S.E.M. of six independent experiments. *, $P = 0.013$ by paired *t*-test. (**c**) Aliquots containing 50 μg (lanes 1, 4), 25 μg (lanes 2, 5), or 12.5 μg (lanes 3, 6) of total cellular protein from untreated cells were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and sequentially probed with rabbit anti-chicken procaspase-6[3] (upper panel) and mouse anti-heat shock protein 90 (a kind gift from David Toft, Mayo Clinic, Rochester, MN) using previously described techniques.[11] (**d**) Cells were treated for 24 h with the indicated concentration of camptothecin or diluent (0.1% DMSO) and examined for the presence of subdiploid cells as described in panel b. Bars, mean ± S.E.M. of three independent experiments. (**e**) After cells were treated for 24 h with 50 nM camptothecin (CPT) or diluent (0.1% dimethyl sulfoxide), cytosol was prepared and assayed for activity capable of cleaving the caspase 3/7-preferred substrate DEVD-AFC and the caspase 6-preferred substrate VEID-AFC as previously described.[12] Bars, mean ± S.E.M. of four independent experiments. (**f**) Cells were treated for 24 h with the indicated investigational anticancer agent at 5 μg/ml (added from a 1000-fold concentrated stock in dimethyl sulfoxide), stained, and examined for subdiploid cells as described for panel b. Flavopiridol (1 μM), a PTEF-b inhibitor[13] that triggers apoptosis by inducing cytochrome c release from mitochondria,[14] was included as an agent that should not be affected by procaspase 6 expression in these assays. Bars, mean ± S.E.M. of four to six independent experiments for various drugs. *, **$P = 0.05$ and $P = 0.03$, respectively, by paired *t*-test. *P*-values in these panels have not been corrected for any effect of multiple comparisons