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Letter to the Editor

Novel survivin-related members of the inhibitor of apoptosis (IAP) family

Dear Editor,

The inhibitor of apoptosis proteins (IAPs) have first been described as baculovirus-encoded proteins that upon infection of insect cells inhibit the host's apoptotic defense mechanisms and enhance viral replication. Recently, several cellular members have been identified in baker's and fission yeast, in the nematode *Caenorhabditis elegans*, the fruitfly *Drosophila melanogaster* and several mammals.[1,2] The common feature of IAPs is that they bear one to three domains of about 70 amino acids in length known as BIRs (baculovirus inhibitory repeats) that inhibit certain caspases and therefore confer anti-apoptotic ability to the cell. Attention was focused on IAP research with the recent discovery of the survivin gene,[3] a human IAP with a single BIR motif. Survivin is expressed in fetal tissues and is not detectable in most adult tissues, but re-expressed in most common human tumors.[4] For several neoplastic diseases such as neuroblastoma, gastric and colorectal carcinoma, the re-expression of survivin has been suggested to be a prognostic factor.[5] Survivin inhibits apoptosis induced by anti-cancer drugs and is able to inhibit CD95- and caspase-induced apoptosis, probably by binding to caspase-3 and -7.[6] In addition to its anti-apoptotic ability, survivin seems to be involved in cell cycle regulation at the G2/M checkpoint.[7,8]

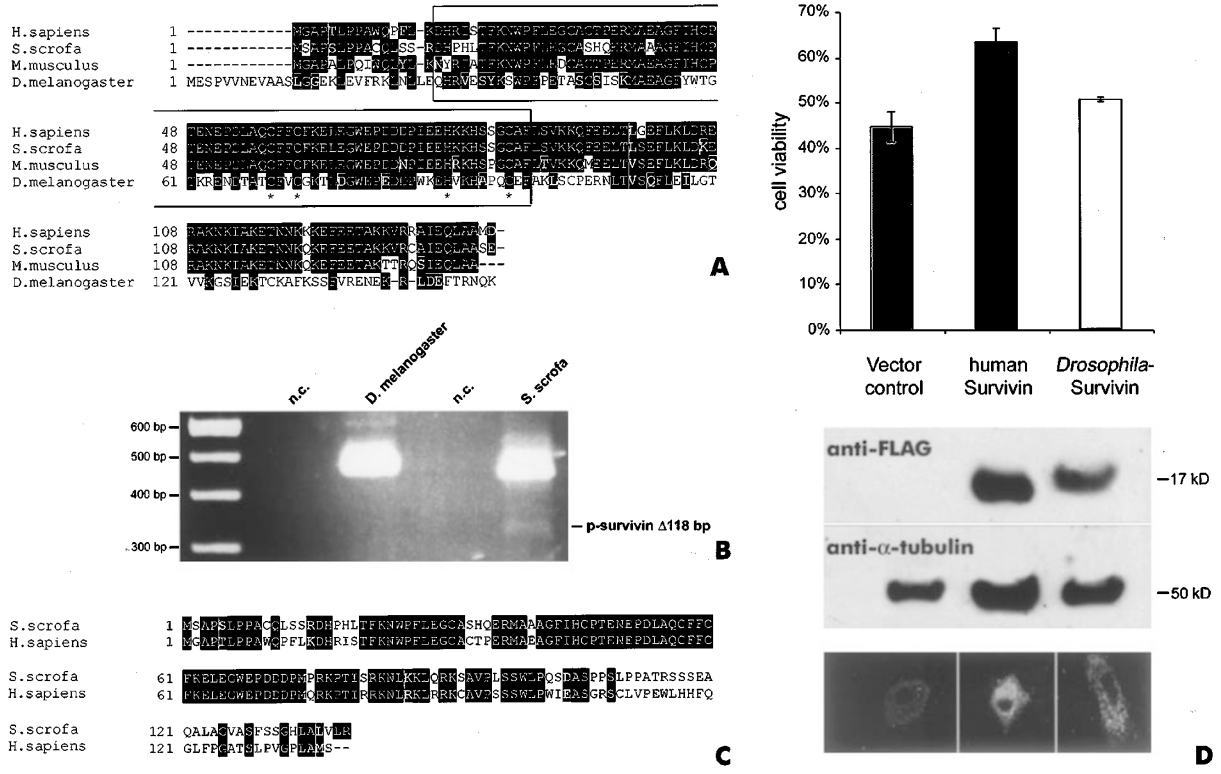

Figure 1 (**A**) Multiple alignment, using ClustalW, of human and murine survivin, and the two novel survivin homologs in *Sus scrofa* and *Drosophila melanogaster*. Residues thought to be critical for BIR function are indicated by an asterisk. Darker shading is of residues that are highly conserved, lighter shading is of less well-conserved residues and residues which are not shaded are not conserved. The BIR domain is boxed. (**B**) Specific RT–PCR confirmed the expression of the proposed survivin variants in RNA samples from *Drosophila* embryos (0–24 h) and adult *Sus* small intestine. In addition, another faint band (=p-survivin-Δ118bp) was observable in the porcine RT–PCR sample (n.c.=negative control). (**C**) Multiple alignment, using ClustalW, of human survivin-ΔEx3 and porcine-Δ118bp protein (shading has been performed in analogy to **A**). (**D**) The anti-apoptotic potential of the *Drosophila* survivin homolog was checked in a mammalian system. Therefore, the coding sequences of *Drosophila* survivin and its human counterpart were ligated into the expression vector pFLAG-CMV-2 and transfected into the human hepatoma cell line HepG2. The transfection efficiency was $\geqslant$50% in all transfection experiments. The percentage of transfected cells was determined by mircoscopic evaluation of β-galactosidase expressing cells. Data are the means$\pm$S.D. of at least three independent experiments. Cell death was induced in transient transfectants by incubation with the agonistic CH11 antibody (500 ng/ml). Immunoblot analysis showed comparable levels of expression for the indicated survivin variants in each transfection assay as evident from comparison with α-tubulin controls. Using mouse anti-FLAG and FITC-labeled anti-mouse antibodies, confocal laser scanning microscopy revealed a mainly cytoplasmatical localization of both human and *Drosophila* survivin protein

We screened the dbEST database for sequences encoding previously unknown IAPs related to survivin. A consensus sequence was deduced with the PROFILE-WEIGHT program using an alignment of the single BIR of human survivin, its murine homolog TIAP[9] and nine metazoan BIRs shown to be homologous to the survivin BIR.[10] Database analysis was then performed by running TBLASTN against the dbEST database. The search produced 112 hits on dbEST entries, 76 of which were further evaluated. The majority of these sequences contained BIR motifs identical to known IAPs, except for five ESTs of non-human/non-murine origin. Two out of these five ESTs, i.e. AF160669 and AI558531, were from the zebrafish *Danio rerio*. AF160669 displayed severe sequencing errors and was, therefore, not evaluated further. The 198 aa protein sequence deduced from AI558531 showed highest identity to murine XIAP. In addition to a complete BIR, two incomplete BIRs at the N- and C-terminus were observable. EST C81977 from the Japanese flounder, *Paralychthys olivaceus*, contained a single open reading frame for a 143 aa protein which closely resembled human cIAP-2, although it contained only a single BIR motif. EST AI260030 from *Drosophila melanogaster* larval-early pupal cDNA and EST AJ241166 from *Sus scrofa* small intestine cDNA contained an open reading frame for a protein with a single BIR motif showing highest identity to human and murine survivin (Figure 1A).

A gene-specific RT–PCR was performed for the *Drosophila* and porcine sequence and confirmed the existence of the proposed mRNAs (Figure 1B). RT–PCR from porcine intestine cDNA showed an additional faint band of 310 bp in length below the expected PCR product of 429 bp. Direct sequencing of the 310 bp product revealed the deletion of a 118 bp portion between Nt 226 and Nt 345 of EST AJ241166, resulting in a frameshift. The deleted sequence showed high identity to human survivin exon 3, resulting in high identity of the encoded protein to the human variant survivin-ΔEx3 (Figure 1C).[11] This observation provides evidence for equal modes of alternative splicing in man and pig. Recently, similar findings on alternative splicing of survivin in mice have been reported.[12] Additionally, the expression of porcine survivin homologs in adult intestine tissue indicates that survivin and its splice variant survivin-Δ118bp may be expressed at the RNA level in non-neoplastic adult tissue.

Because the known IAPs of *Drosophila* were reported to inhibit apoptosis in mammalian cells,[13] we further assessed the anti-apoptotic ability of the *Drosophila* survivin protein in HepG2 hepatoma cells susceptible to apoptosis induction via the CD95 pathway.[14] Therefore, the putative coding sequence was ligated into the mammalian expression vector pFLAG-CMV-2 and transfected into HepG2 cells. Induction of CD95-mediated cell death by trimerization of CD95 receptors with the agonistic CH11 antibody revealed marked differences in the anti-apoptotic activity of human and fruitfly survivin (Figure 1D). Whereas cells transfected with human survivin showed a significantly increased cell survival, cells transfected with the *Drosophila* protein exhibited minor increases in cell survival only. This weak anti-apoptotic potential of the *Drosophila* survivin protein might be explained by a molecular target unique for *Drosophila* and/or a specialized function in cell cycle regulation, which has been observed for human survivin as well[7] but is not detectable by our assay. Interestingly in this context, the program COILS[15] revealed a 14 aa long repeat typical for a coiled-coil domain in the poorly conserved C-terminus of *Drosophila* survivin, suggesting a conserved potential for interaction with components of the mitotic spindle, e.g. microtubuli.

The *Drosophila* survivin homologue identified in this study opens new perspectives to study the role of this unique IAP in cell cycle regulation and apoptosis. The elucidation of distinct mechanisms of interaction with other molecular targets in the fruitfly might also lead to a better understanding of mechanisms controlling cell division or cell death in human disease.

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1. LaCasse EC, *et al.* (1998) Oncogene 17: 3247–3259
2. Deveraux QL and Reed JC (1999) Genes Dev. 13: 239–252
3. Ambrosini G, *et al.* (1997) Nat. Med. 3: 917–921
4. Adida C, *et al.* (1998) Am. J. Pathol. 152: 43–49
5. Altieri DC and Marchisio C (1999) Lab. Invest. 79: 1327–1333
6. Tamm I, *et al.* (1998) Cancer Res. 58: 5315–5320
7. Li F, *et al.* (1998) Nature 396: 580–583
8. Li F, *et al.* (1999) Nat. Cell Biol. 1: 461–466
9. Kobayashi K, *et al.* (1999) Proc. Natl. Acad. Sci. USA. 96: 1457–1462
10. Stehlik C, *et al.* (1998) Biochem. Biophys. Res. Comm. 243: 827–832
11. Mahotka C, *et al.* (1999) Cancer Res. 59: 6097–6102
12. Conway EM, *et al.* (2000) Blood 95: 1435–1442
13. Hawkins CJ, *et al.* (1998) Cell Death Differ. 5: 569–576
14. Müller M, *et al.* (1997) J. Clin. Invest. 99: 403–413
15. Lupas A (1996) Methods Enzymol. 266: 513–525