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Cloning and functional characterization of the guinea pig apoptosis inhibitor protein Survivin

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ABSTRACT

Background: The guinea pig is widely used as a model to study (patho)physiological processes, such as neurodegenerative disorders. Survivin's dual function as an apoptosis inhibitor and a mitotic regulator is crucial not only for ordered development but its modulation seems crucial also under disease conditions. However, data on the expression and function of the guinea pig Survivin protein ($Survivin_{Gp}$) are currently lacking.

Results: Here, we here report the cloning and functional characterization of $Survivin_{Gp}$. The respective cDNA was cloned from spleen mRNA, containing a 426 bp open reading frame encoding for a protein of 142aa. $Survivin_{Gp}$ displays a high homology to the human and murine orthologue, especially in domains critical for function, such as binding sites for chromosomal passenger complex (CPC) proteins and the nuclear export signal (NES). Notably, phylogenetic analyses revealed that $Survivin_{Gp}$ is more related to humans than to rodents. Ectopic expression studies of a $Survivin_{Gp}$-GFP fusion confirmed its dynamic intracellular localization, analogous to the human and murine counterparts. In interphase cells, $Survivin_{Gp}$-GFP was predominantly cytoplasmic and accumulated in the nucleus following export inhibition with leptomycin B (LMB). A typical CPC protein localization during mitosis was observed for $Survivin_{Gp}$-GFP. Microinjection experiments together with genetic knockout demonstrated that the NES is essential for the anti-apoptotic and regulatory role of $Survivin_{Gp}$ during cell division. *In vivo* protein interaction assays further demonstrated its dimerization with human Survivin and its interaction with human CPC proteins. Importantly, RNAi-depletion studies show that $Survivin_{Gp}$ can fully substitute for human Survivin as an apoptosis inhibitor and a mitotic effector. Immunohistochemistry, immunofluorescence, and western blotting were employed to detect Survivin expression in guinea pig tissues. Besides its expression in proliferating tissues, such as spleen and liver, we also found Survivin in terminally differentiated cell types. Importantly, Survivin was detectable also in the cochlea, suggesting a potential role for Survivin in the auditory system.

Conclusions: We provide the first experimental evidence for the expression of Survivin in the guinea pig. As $Survivin_{Gp}$ can substitute for known functions of human Survivin, the guinea pig model will now also allow investigating Survivin's (patho)physiological role and to test Survivin-directed potential therapeutic strategies.

1. Introduction

Animal models have aided in the identification of factors and molecular circuitries involved in development, aging, and disease. Also, the guinea pig model is used as a clinically relevant facsimile of human diseases, particularly in the area of hearing research (Canlon et al., 2007; Bahekar et al., 2008). However, the current lack of molecular tools represents a bottleneck to fully exploit the potential of this animal model. In particular, disease patterns and therapeutic intervention strategies often involve the rational modulation of mitotic or apoptotic processes (Fadeel and Orrenius, 2005; Canlon

Abbreviations: α, anti; aa, amino acids; Ab, antibody; BCL-2, B-cell lymphoma 2; BIR, baculovirus IAP repeat; bp, base pairs; CPC, chromosomal passenger complex; CRM1, chromosomal region maintanance; GFP, green fluorescent protein; Gp, guinea pig; GST, glutathione S-transferase; Hu, human; IAP, inhibitor of apoptosis protein; INCENP, inner centromere protein; IHC, immunohistochemistry; LMB, leptomycin B; mut, mutant; NES, nuclear export signal; NLS, nuclear import signal; PCD, programmed cell death; RING, Really Interesting New Gene; RNAi, RNA interference; scr, scrambled control; siRNA, small interfering RNA; wt, wild type.

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et al., 2007; Youle and Strasser, 2008). Deregulation of these processes culminating in cell loss, include stroke, neurodegeneration and hearing impairment research (Canlon et al., 2007; Bahekar et al., 2008), or disease characterized by a failure to eliminate harmful cells like cancer and autoimmunity (Fadeel and Orrenius, 2005).

In general, modulation of programmed cell death (PCD) can be achieved *inter alia* by the dynamic expression of pro- and anti-apoptotic BCL-2 protein family members as well as of apoptosis inhibitor proteins (IAP) (Stauber et al., 2007; Altieri, 2008; Youle and Strasser, 2008).

In humans, the *Survivin* gene on chromosome 17q25 potentially gives also rise to four alternatively spliced transcripts (Li, 2005). However, not all variants have been unambiguously shown to be transcribed or even expressed *in vivo*, and there are conflicting reports concerning their potential biological functions (Li, 2005; Noton et al., 2006; Knauer et al., 2007a). Human wild type Survivin (16.5 kDa), the smallest member of the IAP family, comprising of 142 amino acids, is characterized by a single baculovirus IAP repeat (BIR), a carboxy-terminal coiled-coil domain, the absence of a carboxy-terminal RING finger domain, and appears to exist as a homodimer (Sun et al., 2005). Survivin is expression is low in the majority of non-malignant interphase cells, whereas there is a pronounced upregulation of Survivin during the G2/M phase of the cell cycle (Lens et al., 2006; Altieri, 2008). Survivin is one of the chromosomal passenger complex (CPC) proteins and interacts with Aurora-B kinase, Borealin and the inner centromere protein (INCENP) in order to execute essential roles during cell division (Lens et al., 2006; Ruchaud et al., 2007). In interphase cells, Survivin seems to inhibit apoptotic executors, e.g., caspases, due to its cytoplasmic localization (Knauer et al., 2007c). It is actively exported into the cytoplasm as Survivin contains a canonical nuclear export signal (NES) interacting with the transport receptor CRM1 and the RanGTP/GDP axis (Knauer et al., 2007c; Stauber et al., 2007; Connell et al., 2008).

Survivin expression is critical for normal embryonic development (Uren et al., 2000). Furthermore, Survivin is highly expressed in most human tumors, and expression appears to correlate with increased resistance to cancer therapy (Stauber et al., 2007; Altieri, 2008; Mehrotra et al., 2010). Notably, recent evidence suggests that Survivin is also expressed in non-malignant tissues, potentially executing cytoprotective functions against various stress conditions (Johnson et al., 2005; Fukuda and Pelus, 2006; Kindt et al., 2008).

Although Survivin is under intense investigation in human medicine, comparatively little is known regarding its expression and molecular function in mammalian animal models except mouse. Consequently, we here present the cloning and functional characterization of the guinea pig Survivin and performed a functional comparison with the human orthologue. Our results indicate that also the guinea pig model is applicable to study the (patho) physiological functions of Survivin.

2. Results

2.1. Cloning of the guinea pig Survivin cDNA

For cloning, we generated cDNA from guinea pig spleen tissue and subjected it to PCR amplification steps using primers, which were predicted to bind to highly conserved sequences in *Survivin* genes from mammals (Additional files 1 and 3). In total, we analyzed six partially overlapping regions by means of "cDNA walking." Sequence analysis finally revealed an open reading frame showing 86% nucleotide identity to the human orthologue, encoding for a protein of 142aa (Fig. 1A, and Additional file 1; GenBank accession number: GQ496319). The Survivin$_{Gp}$ protein displays a high homology to the human and murine orthologue, especially in domains critical for function, such as the nuclear export signal (NES), protein interaction domains, and posttranslational modification sites (Ruchaud et al., 2007; Stauber et al., 2007; Altieri, 2008) (Fig. 1A). Sequence comparison with Survivin from other species in terms of amino acid conservation (Additional file 1) as well as in form of a phylogenetic tree (Fig. 1B), revealed that despite its evolutionary affiliation to the rodents, Survivin$_{Gp}$ shows a higher similarity to the human than to the murine counterpart (Fig. 1A). As the expression of human and mouse Survivin splice variants in cancer cells has been shown on the mRNA level, we performed RT-PCR to examine the presence of Survivin$_{Gp}$ splice forms in adult guinea pig tissues. We could only detect a PCR product corresponding to wt *Survivin$_{Gp}$* and no additional bands indicative of the expression of *Survivin$_{Gp}$* isoforms were detectable in the spleen, heart or cochlea (Additional file 1D, and data not shown). Hence, it can be assumed that if expressed at all, the guinea pig Survivin variants appear to be expressed at very low levels.

2.2. The Survivin$_{Gp}$ localizes as a typical CPC protein capable of interacting with human CPC members

To compare the functional properties of the guinea pig Survivin protein with those of its human homologue, we first examined its localization during mitosis. In HeLa cells transiently expressing Survivin$_{Gp}$-GFP, immunofluorescence analysis revealed that Survivin$_{Gp}$-GFP correctly localized during mitosis, i.e., at the centromeres from pro- to metaphase, at the spindle midzone during anaphase and at the midbody during telophase and cytokinesis (Fig. 2; Additional file 2A). Survivin's mitotic functions critically depend on its interaction with the other CPC members, which is at least partially reflected by their correct colocalization (Vagnarelli and Earnshaw, 2004; Knauer et al., 2006). Indeed, the human CPC proteins AuroraB kinase, Borealin and INCENP colocalized with Survivin$_{Gp}$-GFP as know for human Survivin (Fig. 2B–D; Additional file 2A). Immunoprecipitation experiments further verified complex formation between Survivin$_{Gp}$-GFP and the human CPC members (data not shown). Hence, we concluded that Survivin$_{Gp}$-GFP is capable of interacting with human CPC members and can assemble in a functional CPC requested to guide cells through mitosis.

As Survivin dimerization appears to be another criterion required for biological function, we applied our translocation-based protein interaction assay to probe heterodimer formation of Survivin$_{Gp}$ with Survivin$_{Hu}$ in living cells (Knauer et al., 2005b). Fluorescence microscopy shows that Survivin$_{Gp}$-GFP is a predominantly cytoplasmic in interphase cells, and its localization nicely concurs with that of human Survivin (Survivin$_{Hu}$; Fig. 3A). In contrast, Fig. 3B illustrates that the cytoplasmic Survivin$_{Gp}$-GFP prey is tethered to the nucleolus upon coexpression of the nucleolar anchored Survivin$_{Hu}$-RevBFP bait (Fig. 3B, upper panel). Similar results were obtained upon coexpression of the cytoplasmic Survivin$_{Gp}$-GFP prey with the Survivin$_{Hu}$-RevBFP bait (data not shown). As a control, no colocalization was observed upon co-expression of Rev-BFP only (Fig. 3B, lower panel), confirming the assay's specificity. Also, Survivin$_{Gp}$-GFP is capable of interacting with the human isoform Survivin3B$_{Hu}$, as ectopic expression of Survivin3B$_{Hu}$-RevBFP results in their colocalization at the nucleolus (middle panel).

2.3. The biological function and localization of Survivin$_{Gp}$ depend on its active nuclear export signal (NES)

Previously, we showed that the functionality of a CRM1-dependent NES in human and murine Survivin is essential for its localization and function as an apoptosis inhibitor and mitotic effector (Knauer et al., 2006; Stauber et al., 2006). However, whether such a requirement is also true for Survivin orthologs from other species has not been examined. First, to demonstrate that also the localization of Survivin$_{Gp}$ depends on the NES/CRM1 interaction, interphase cells showing cytoplasmic Survivin$_{Gp}$-GFP were treated with the export inhibitor leptomycin B (LMB), resulting in the nuclear accumulation of