The role of survivin in angiogenesis during zebrafish embryonic development

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Abstract

Background: Survivin is the smallest member of the inhibitor of apoptosis (IAP) gene family containing a single Baculovirus IAP Repeat (BIR) domain and an extended COOH termin-al α-helical coiled coil [1]. Survivin is not expressed in most normal adult tissues but is highly expressed in solid and hematological malignancies, where it has been linked to increased angiogenesis and tumorigenesis [2,3]. During

Published: 18 May 2007
BMC Developmental Biology 2007, 7:50 doi:10.1186/1471-213X-7-50
Received: 15 October 2006
Accepted: 18 May 2007

This article is available from: http://www.biomedcentral.com/1471-213X/7/50
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human and murine embryonic development, survivin is ubiquitously expressed [4]. However, homozygous knock-out of survivin in mouse ES cells results in disrupted microtubule formation and polyploidy as well as early embryonic fatality, precluding characterization of its functions during murine development [5]. As a result, the role of survivin during embryonic development remains unclear.

Recently, the zebrafish survivin-1 gene (abbreviated survivin) has been cloned, showing remarkable sequence identity and similarity over the BIR domain compared with human and mouse survivin gene [6]. Microarray analysis showed that survivin is significantly up-regulated in a zebrafish chordin morphant in which the intermediate cell mass (ICM, where vascular and primitive hematopoietic tissues arise) was expanded [7]. Here, we investigated if survivin plays a role in vascular formation during zebrafish embryonic development.

Results

Expression of survivin in zebrafish embryos

Whole-mount in-situ hybridization was performed to examine survivin mRNA expression in zebrafish embryos at 26 hpf. Survivin was detected diffusely throughout the developing brain and neural tube. It was also expressed at the vicinity of the axial vasculature from which the inter-segmental vessels arise (Figure 1a–b). This was further confirmed in histological sectioning in which the areas corresponding to the developing axial vasculature and neural tube showed increased expression relative to the adjacent tissues (Figure 1b, insert). Furthermore, double in-situ hybridization showed that survivin was expressed in the developing axial vasculature dorsal to the intermediate cell mass (ICM), where gene encoding for embryonic hemoglobin \( \alpha \) was expressed. The pattern was remarkably similar to that of flk1, a VEGF receptor tyrosine kinase (Figure 1c–d).

Survivin morphants

The role of survivin during embryonic development was investigated by knocking-down its function using MOs. The phenotypic penetrance of survivin MOs was dose- and time-dependent. At 22 hpf, when injected with either 3 ng SurvITR or 3 ng SurvATG-MOs (referred as SurvITR\textsuperscript{mo} and SurvATG\textsuperscript{mo} embryos), most embryos had a relatively normal morphology (Figure 2a,c). However, at 48 hpf, 74.8 ± 7.3% and 72.0 ± 4.0% of embryos manifested "characteristic phenotypes" with reduced eye and head sizes and a mildly curved tail (Figure 2b,d). There were no overt tissue necrosis in these embryos. At 6 ng of either MOs, increasing numbers of embryos became severely deformed and died shortly after 48 hpf (Figure 2d, insert). Co-injecting SurvATG + SurvITR-MOs (3 ng each) resulted in specific phenotypes in 79.4 ± 7.2% embryos without increase in toxicity or mortality as compared with 3 ng of either MO alone (Figure 2e). The combination regimen remained significantly less toxic than that of SurvITR-MO at 6 ng. In all subsequent experiments, SurvATG and SurvITR-MOs were co-injected at 3 ng each (referred as SurvITR-ATG\textsuperscript{mo} embryos). Only embryos with characteristic phenotypes were investigated while those which were severely deformed were excluded from analysis.

Effects of survivin knock-down on angiogenesis

We have previously shown that survivin is significantly up-regulated in a zebrafish chordin morphant in which the ICM was expanded [7]. Therefore, we first examined the effects of survivin knock-down on vascular formation in Tg(fli1:EGFP)\textsuperscript{y1} embryos. In uninjected embryos, the axial circulation (AC), inter-segmental vessels (ISV), dorsal longitudinal anastomotic vessels (DLAV), vertebral and sub-intestinal vessels (SIV) were readily observable (Figure 3a,c). In SurvITR-ATG\textsuperscript{mo} embryos, the dorsal aorta and posterior cardinal vein were patent, indicative of intact vascularogenesis (see additional file 1: Wild-type embryos and file 2: Survivin morphants). However, the development of vertebral and ISV was perturbed with defective or total absence of sprouting as well as failure to form the DLAV and SIV (Figure 3b,d). These defects were seen in all 54 SurvITR-ATG\textsuperscript{mo} embryos observed (\( n = 3 \) separate experiments) with the characteristic phenotypes. The results were confirmed using microangiography in which defective ISV sprouting and failure to form the DLAV, as well as defective inner optic circle (IOC) and optic veins (OV) of the developing eyes were seen in the SurvITR-ATG\textsuperscript{mo} embryos (Figure 3e–h). Similar patterns of angiogenesis defects were observed when either SurvITR or SurvATG morpholinos were injected (data not shown).

Effects of survivin MOs on apoptosis as shown by TUNEL and caspase-3 activity

As a member of the IAP family, survivin has been shown to inhibit apoptosis by regulating caspase activity [1,2]. Therefore; we investigated if there was increased apoptosis in the SurvITR-ATG\textsuperscript{mo} embryos as measured by TUNEL assay. At both 24 and 48 hpf, increased TUNEL staining was detected in the developing neural tube and the brain (not shown), with significant, albeit weaker, staining at the vicinity of the axial vasculature (Figure 3i,j). The increased apoptosis was further confirmed by specific caspase-3 activity which was significantly increased in 48 hpf SurvITR-ATG\textsuperscript{mo} embryos (299.1 ± 8.3 arbitrary units) compared with control embryos injected with a random sequence MO at 6 ng (103.0 ± 2.3 arbitrary units, \( n = 3 \) experiments using 240 embryos, \( p < 0.05 \)).

Specificity of survivin knock-down

To further demonstrate the efficacy of SurvITR and SurvATG MO binding to survivin mRNA, embryos were co-injected
with a 5'UTR survivin:GFP plasmid (50 pg) and SurvITR/ATG-MOs (3 ng each). Injecting the plasmid alone lead to GFP expression in 79.7 ± 9.4% embryos (Figure 4a,c). Co-injection of the plasmid with SurvITR/ATG-MOs completely abolished protein translation and hence GFP expression in all embryos tested (Figure 4b,d). A splice site MO (SurvSS-MO (12 ng)) not only induced similar morphological changes as in SurvITR/ATG-mo embryos (smaller head and eye size and mildly curved tail) but also induced defective angiogenesis as shown in Tg(fli1:EGFP)y1 embryos (61.7%, n = 3 experiments using 159 embryos) (Figure 4e–f). Angiogenesis defects were seen in ISV as well as OV/IOC of the developing eyes (not shown). A relatively high dose of MO (12 ng) was used as lower doses...
produced less phenotypic penetrance and at 12 ng, there was no excessive mortality. In the Sur_{33}^{MO} embryos, RT-PCR confirmed defective splicing of part of the intron, as shown by a larger PCR transcript which was verified by bi-directional DNA sequencing (Figure 4g,h). Whether defective splicing could be induced by lower doses of this MO has not been examined. Finally, defective sprouting or failure to form the DLAV occurred in all Sur_{UTR+ATG}^{MO} embryos and co-injecting survivin mRNA (30 pg) with Sur_{UTR+ATG}^{MO} MOs rescued the vascular defect in 47 out of 58 embryos in three separate experiments (81%) (Figure 4i–l).

**Effects of VEGF on survivin expression**

VEGF plays an important role in angiogenesis during zebrafish embryonic development [8]. *In-vitro* studies have shown that survivin mediates the proliferative and anti-apoptotic effects of VEGF in endothelial cells [9]. Therefore, we investigated if survivin expression during embryogenesis is regulated by VEGF. Exogenous human VEGF protein (2 ng) was injected into zebrafish embryos at one-cell stage [10]. Angiogenesis was examined in the sub-intestinal vessels at 96 hpf, where the vasculature was well-developed and any ectopic structures could be readily detectable. In 78 out of 110 embryos (70%) (from three separate experiments), VEGF induces ectopic angiogenesis which was associated with a significant up-regulation of survivin mRNA expression (Figure 5a–b,f). We also incubated embryos with a VEGF receptor inhibitor (VEGFTKR) at one-cell stage. VEGFTKR (25 μmol/L) induced defective angiogenesis in all treated embryos at 48 hpf (Figure 5c–d) and could not be rescued by survivin mRNA injection (30 pg) (Figure 5e). Survivin mRNA expression was significantly down-regulated in these embryos (Figure 5f).

**Discussion**

In this study, we demonstrated that knock-down of survivin with MOs gives rise to embryos with reduced eye and head sizes and a mildly curved tail. Similar phenotypic changes have been described in a survivin mutant generated in a retrovirus insertional mutagenesis screen [11]. The survivin morphants had defective angiogenesis but vasculogenesis, i.e. formation of axial vasculature, was not affected at the doses of MOs tested. Development delay in these morphants was not observed, as shown by the normal onset and pattern of pigmentation and heart beat (data not shown). Our results corroborate with *in-vitro* studies showing that survivin is important for the maintenance of proliferation and survival on endothelial cells [9,12]. In addition, our data provided new information on the role of survivin during embryonic development.

*In-vitro* and tumorigenesis studies have shown that survivin mediates the angiogenic effects of VEGF [9,12,13]. In zebrafish embryos, VEGF signaling is important for angiogenesis. In particular, mutants defective in a
zebrafish orthologue of flk1 (a VEGF-receptor tyrosine kinase), the schwentine [14], and in phospholipase Cγ (a tyrosine kinase mediating effects of VEGFR), the y10 [15], exhibit specific defects in angiogenesis. MO targeting of VEGF results in defective circulation in the head, axial and inter-segmental vasculature in a dose-dependent fashion [8]. In this study, VEGF induces ectopic angiogenesis and up-regulates survivin mRNA expression, suggesting that survivin may mediate the angiogenic effect of VEGF. The link between VEGF and survivin during zebrafish angiogenesis has not been examined but may involve PKB/Akt signaling as reported in human endothelial cell lines [16]. Intriguingly, co-injecting embryos with survivin mRNA could only rescue the vascular defects seen in SurUTRATC-MO embryos but not in embryos treated with a VEGF receptor inhibitor. Therefore, additional downstream mediators may be involved in the angiogenic effects of VEGF [17]. Reversely, whether VEGF can rescue the angiogenesis defects in SurUTRATC-MO embryos has not been examined. Perturbation of VEGF signaling may also result in changes in blood vessel synthesis and the observed changes in survivin mRNA may reflect changes in endothelial cell number rather than a direct mechanistic link to VEGF signaling. This issue would have to be evaluated in future study.

Both human and murine studies have demonstrated that survivin is involved in haematopoietic stem and progenitor cell proliferation [18,19]. However, in the present study, early specification of hematopoietic progenitors in the SurUTRATC-MO embryos was not affected, as shown by the normal expression of genes encoding for hematopoietic transcription factors and embryonic hemoglobins, as well as the normal distribution of gata1 + population in Tg(gata1:GFP) embryos at 18 hpf, before the onset of functional circulation (data not shown).

That the targeting of the survivin MOs was specific was shown using several control studies. First, the phenotypic changes seen in Sur UTR+ATGMO embryos were similar to those observed in survivin mutants generated by retrovirus insertional mutagenesis screening [11]. Indeed, it would be valuable to examine this mutant for similar defects in angiogenesis. Second, co-injecting SurUTR+ATG-MO with a 5'UTR-survivin:GFP plasmid inhibited translation and hence green fluorescence induced by the latter in all embryos, proving efficacious binding of SurUTR+ATG-MO to the 5'UTR-survivin mRNA. Third, the angiogenesis defects of ISV induced by SurUTR+ATG-MO could be rescued by survivin mRNA. Whether the defects in SIV and OV/IOC were similarly reversed and whether injection of survivin mRNA alone would induce angiogenesis defects would have to be further examined. Finally a splice-site morpholino recapitulated the phenotypes seen with SurUTR+ATG-MO. Therefore, the angiogenesis defects in SurUTR+ATGMO embryos represent a specific phenotype due to knock-down of survivin function in zebrafish embryos.

In human, murine [4] as well as Xenopus embryos [20], survivin is ubiquitously expressed. These observations, together with the fact that the developing head and eye of the SurUTRATC-MO embryos were reduced in size have...
Regulation of survivin expression by vascular endothelial growth factor (VEGF) at 96 hpf. (a): Sub-intestinal vessels in uninjected Tg(fli1:EGFP)y1 embryos. (b): Injection of human VEGF (2 ng) gave rise to ectopic angiogenesis (arrows). There was no observable ectopic angiogenesis in the ISV (c): Axial and inter-segmental vessels in untreated Tg(fli1:EGFP)y1 embryos. (d): Tg(fli1:EGFP)y1 embryos treated with VEGF tyrosine kinase receptor inhibitor (VEGFTKRI, 25 μmol/L) showing defective sprouting of inter-segmental vessels. (e): Injection of survivin mRNA expression (expressed in fold-change) in untreated and VEGFTKRI treated embryos as well as in embryos injected with human VEGF. Results expressed in mean ± S.E.M. (n = 3 experiments using at least 20 embryos per experiments). When the three groups of data were compared using Kruskal-Wallis Test, p-value = 0.016. When the data of uninjected vs VEGFTKRI treated embryos were compared using Mann-Whitney U Test, p-value = 0.037.

Several observations in this study have remained unexplained. For instance, we did not observe a direct causal link between increased apoptosis and the angiogenesis defect in the SurUTR+ATGMO embryos. Apoptosis was detectable not only in the axial vasculature, but also in the developing brain and neural tube of the SurUTR+ATGMO embryos. Both in-vivo and in-vitro studies have demonstrated that in addition to its anti-apoptotic function, survivin plays an important role in the regulation of cellular proliferation and cytokinesis [1,2]. Recent study in Xenopus embryos also showed that survivin expression induces endothelial cell proliferation independent of apoptosis [20]. Therefore, the relative modest TUNEL staining in the axial vasculature did not preclude the role of survivin in angiogenesis. It is also possible that survivin plays a non-cell autonomous role in the angiogenesis process. Childs et al. (2002) [21] demonstrated in zebrafish embryos the migration of angioblasts from the aorta to the dorsal aspect of the neural tube and to the inter-phase between notochord and the somites, where they develop into DLAV and ISV. Therefore, vascular patterning may depend on signaling cues that direct the site of angiogenesis sprouts. Whether the occurrence of apoptosis in the developing neural tube might have perturbed these signals hence the formation of DLAV and ISV would have to be carefully examined. The proposition may also explain the lack of robust expression at the site of ISV and DLAV in wild-type embryos. Furthermore, although survivin is expressed robustly in the axial vasculature, concomitant expression was noted in the developing central nervous system. The expression of survivin within these structures needs to be defined in future study. Moreover, the developing eye and head structures in the survivin morphants are generally smaller. Whether this reflected changes secondary to defective angiogenesis or alternative functions of survivin during development have not been elucidated. Finally, survivin gene in zebrafish has undergone duplication during evolution [22] and the function of the duplicated gene would have to be further investigated. Notwithstanding these limitations, our data still support the proposition that survivin is involved in the regulation of angiogenesis during zebrafish development.
Survivin is strongly expressed in both solid organ and hematological malignancies where it is associated with treatment failure and a poor prognosis [2,3]. Loss of function studies have also demonstrated that survivin expression is linked to angiogenesis and tumorigenesis in gastric and colonic cancers and has become a potential target for anti-cancer therapy [23,24]. Our observation that survivin regulates angiogenesis in zebrafish embryos highlights the relevance of using zebrafish embryos in the screening for survivin-based anti-cancer agents.

In summary, we demonstrate that survivin plays an important role in angiogenesis during embryonic development and may be one of the down-stream effectors of VEGF signaling. Early hematopoiesis was not affected but the role of survivin during late hematopoiesis remains to be determined.

**Methods**

**Zebrafish and morpholinos**

Wild-type zebrafish (*Danio rerio*) were obtained from local aquarium and were maintained and raised under standard conditions at 28°C. Transgenic Tg(fli1:EGFP);1 embryos were used to track endothelial cell populations. Anti-sense morpholinos (MO) (Gene-Tools, OR, USA) were designed to target the 5’ untranslated region (UTR) or sequences flanking and including the initiation codon (ATG) of the zebrafish survivin gene. A splice-site (SS) MO was designed to target the exon2-intron junction of the survivin gene (SurSS-MO). A random sequence MO was used as a control as described previously (Table 1). Procedures for micro-injection, whole mount in-situ hybridization, microangiography, TUNEL and caspase-3 activity assays have been described previously [7,25,26].

**Double in-situ hybridization**

Wild-type (WT) embryos at 24 hpf were fixed with 4% paraformaldehyde (PFA) and dehydrated. After stepwise re-hydration, the embryos were incubated in pre-hybridization buffer (50% formamide, 5 × SSC, 50 μg/ml heparin, 0.1% Tween20, 5 mg/ml rRNA in phosphate-buffered saline, PBS) at 65°C followed by overnight incubation with digoxigenin (DIG)- (either flk-1 or survivin-1) and fluorescein-labeled riboprobe (α-embryonic globin) at 65°C. The embryos were washed and incubated with alkaline phosphatase (AP) conjugated anti-DIG antibody (Roche Molecular Biochemicals, Mannheim, Germany) for overnight at 4°C. Blue color was developed using NBT/BCIP (Roche Molecular Biochemicals, Mannheim, Germany) as substrate and the reaction was stopped with 0.5 mM EDTA in PBT. AP was destroyed by washing the stained embryos with 0.1 M glycine-HCl, pH 2.2 in PBT for 10 min twice. Background staining was removed by washing the embryos in absolute ethanol with continuous monitoring. After re-hydration to PBT, embryos were incubated with AP conjugated anti-fluorescein antibody.

**Table 1: Sequences of oligos used.**

| Oligo                          | Sequence                              |
|-------------------------------|---------------------------------------|
| Morpholinos                   |                                       |
| Su_ATG                        | TGC AAG ATC CAT TTT GTG GGA GGT T     |
| Su_UTR                        | GTG GAA ATT AAA CAA AAG ACA ACC G     |
| Su_SS                         | AGA CAC GGA CTC ACT CAG GGT CAT C     |
| Random Sequence               | CCT CTT ACC TCA GTT ACA ATT TAT A     |
| **Primers for the cloning of survivin mRNA in riboprobe synthesis** |                                       |
| ZF SUR1                       | GCG GAT TTA TCT CGG TTG TCT           |
| ZF SUR1R                      | CAA CTT TCA CAA GTG ACA GAA CAC       |
| **Primers for the cloning of survivin UTR for 5’UTR survivin-GFP construct synthesis** |                                       |
| ZF SUR UTR1                   | GCG GAT TTA TCT CGG TTG TCT           |
| ZF SUR UTR2                   | CAA CTT TCA CAA GTG ACA GAA CAC       |
| **Primers for RT-PCR for survivin mRNA in splice-site morpholino study** |                                       |
| ZF SUR1                       | GCA CTC CCC ACA AAA TGG AT            |
| ZF SUR1R                      | GTC CAC AGT CTT CTT CAG CA            |
| **Primers for the cloning of survivin mRNA in rescue experiments** |                                       |
| ZF SUR1                       | AAT CAA CAA GCA AGCGAG AC             |
| ZF SUR1R                      | CAA TTT ATT AAG CCC GAA TGC           |
| **Primers for real-time quantitative RT-PCR for survivin mRNA** |                                       |
| ZF SUR1                       | CAC TCC AGA AAA CAT GGC TAA A         |
| ZF SUR1R                      | CCA TCC TTC CAG CTC TT A              |
for comparison.

of embryos and were exposed to equal volume of DMSO. Control experiments were set up from the same clutches hpf with continuous exposure to inhibitors until 48 hpf. One-cell stage onwards. They were dechorionated at 24 μmol/L (stock solution in DMSO at 10 mmol/L) from one-cell stage onwards. They were dechorionated at 24 hpf with continuous exposure to inhibitors until 48 hpf. Control experiments were set up from the same clutches of embryos and were exposed to equal volume of DMSO for comparison.

Synthesis of anti-sense mRNA riboprobe for survivin

The full length sequence of zebrafish survivin including the 3' UTR was amplified by PCR (Table 1) from cDNA of 24 hpf embryos and subcloned into pGem-T vector (pGEM-T Vector Systems, Promega, Madison, WI, USA). A 623 bp anti-sense survivin mRNA riboprobe was synthesized from linearized vector containing the insert. A digoxigenin labeled mRNA probe was synthesized by SP6 RNA polymerase according the manufacturer's protocols (Roche Applied Science, Indianapolis, IN, USA). The size and integrity of the synthesized riboprobe was confirmed by RNA formaldehyde gel electrophoresis. Histological assessment of stained embryos was performed on 5–7-μm paraffin sections.

Construction of 5'UTR-survivin:GFP plasmids

The 5'UTR of survivin, including the target sequences of Sur5'UTR-MO and Sur3'UTR-MO, were amplified from 24 hpf wild-type embryo cDNA (Table 1). PCR products were gel purified and cloned in frame and immediately upstream of the GFP coding sequence into vector pGEM3.1/CT-GFP-TOPO (Invitrogen, Carlsbad, CA, USA) and transformed into chemically competent E. coli TOP10 (Invitrogen, Carlsbad, CA, USA). Plasmids containing the 5'UTR-survivin:GFP fusion sequence were isolated and the sequence of the DNA inserts verified using the GFP reverse primer (5'GGG TAA GCT TTC CGT ATG TAG C-3').

Preparation of survivin mRNA for rescue experiments

The complete coding sequence of survivin was TA-cloned into pGEM-T vector (pGEM-T Vector Systems, Promega, Madison, WI, USA) and the orientation of the insert confirmed by PCR (Table 1). mRNA transcripts were synthesized from the T7 promoter of the Sal I digested pGEMT-Sur sequence using the mMessage mMachine Kit (Ambion, Austin, TX, USA).

Treatment of embryos with VEGF receptor tyrosine kinase inhibitors

Embryos were treated with an inhibitor of vascular endothelial growth factor receptor tyrosine kinase (VEGF-FRTK inhibitors, Calbiochem, EMD Bioscience, CA, USA). The embryos were incubated in inhibitor solution at 25 μmol/L (stock solution in DMSO at 10 mmol/L) from one-cell stage onwards. They were dechorionated at 24 hpf with continuous exposure to inhibitors until 48 hpf. Control experiments were set up from the same clutches of embryos and were exposed to equal volume of DMSO for comparison.

Vascular endothelial growth factor (VEGF) injection

Human VEGF protein (BD Bioscience, Bedford, MA, USA) was prepared in 1 mg/mL in water. Embryos at 1–4 cell stage were injected with VEGF (2 ng) into the yolk sac and its effect on angiogenesis was examined at 96 hpf.

Real-time quantitative RT-PCR (Q-PCR)

cDNA from 48 and 96 hpf embryos were reverse transcribed from RNA and Q-PCR for survivin was performed using the SYBR green PCR master mix (Applied Biosystems, Foster City, CA, USA). Expression level was presented as fold-change calculated using the comparative C_T method as described [27] with β-actin as the internal reference. Primer sequences for Q-PCR were shown in Table 1.

Statistical analysis

Results were expressed as mean ± SEM unless otherwise stated. Comparisons between groups of data were evaluated by Mann-Whitney U and Kruskal-Wallis Test where appropriate. P-value of less than 0.05 was considered statistically significant.

Authors' contributions

ACHM carried out the microinjection and molecular studies and wrote the manuscript. RL carried out the microinjection in some experiments. PKC carried out the confocal microscopy. JL and LC performed the histological sectioning of embryos. AM, CV and RL participated in the design and coordination and wrote the manuscript. All authors read and approved the final manuscript.

Additional material

Additional File 1

Blood circulation in wild-type embryos. In wild-type embryos, normal axial circulation is observed as well as circulation in ISV and DLAV. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-213X-7-50-S1.mov]

Additional File 2

Blood circulation in survivin morphants. In survivin morphants, normal axial circulation is observed but circulation in ISV and DLAV was absent. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-213X-7-50-S2.mov]

Acknowledgements

We would like to thank Dr. Stephen C Ekker, Department of Genetics, Cell Biology and Development, University of Minnesota, MN, USA for his helpful discussion and comments on the manuscript. We also thanked Dr. S.H. Cheng and the Confocal Facility in City University of Hong Kong for focal...
microscopy. Thanks are extended to Jessie Fu, Bab Kwok and Tommy Kwan for performing some of the microinjection experiments and to Mr. Howard Chow for part of the molecular studies.

This work was supported by RGC grant (HKU 7488/04M and 7520/06M) and small project funding from CRCG (HKU).

References

1. Altieri DC: Molecular circuits of apoptosis regulation and cell division control: The survivin paradigm. J Cell Biochem 2004, 92(6):656-663.
2. Altieri DC: Validating survivin as a cancer therapeutic target. Nature Reviews Cancer 2003, 3:46-54.
3. Graaf AO, de Witte T, Jansen JH: Inhibitor of apoptosis proteins: new therapeutic targets in hematological cancer? Leukemia 2004, 18:1751-1759.
4. Adida C, Cropp P, Berrebi MD, Diebold J, Altieri DC: Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. Am J Pathol 2003, 162:43-49.
5. Uren AG, Wong L, Paskusch M, et al.: Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. Curr Biol 2000, 10(21):1319-28.
6. Inohara N, Nuliez G: Genes with homology to mammalian apoptosis regulators identified in zebrafish. Cell Death Diff 2000, 7:509-510.
7. Leung AY, Mendenhall EM, Kwan TT, et al.: Characterization of expanded intermediate cell mass in zebrafish chordin morphants. Dev Biol 2005, 277(1):235-54.
8. Nasevicius A, Larson J, Ekker SC: Distinct requirements for zebrafish angiogenesis revealed by a VEGF-A morphant. Yeast 2000, 17(4):294-301.
9. Mesri M, Morales-Ruiz M, Ackermann EJ, et al.: Suppression of vascular endothelial growth factor-mediated endothelial cell protection by survivin targeting. Am J Pathol 2001, 158(5):1757-1765.
10. Serbedzija GN, Flynn E, Willett CE: Zebrafish angiogenesis: A new model for drug screening. Angiogenesis 1999, 3:353-359.
11. Fukudaram A, Nishizawa R, Sun Z, Swindell EC, Farrington S, Hopkins N: Identification of 315 genes essential for early zebrafish development. Proc Natl Acad Sci USA 2004, 101(35):12792-7.
12. Tran J, Rak J, Sheehan C, et al.: Marked induction of the IAP family antiapoptotic proteins survivin and XIAP by VEGF in vascular endothelial cells. Biochem Biophys Res Commun 1999, 264:781-788.
13. Beierle EA, Nagaram A, Dai W, Iyenger M, Chen MK: VEGF-mediated survivin expression in neuroblastoma cells. Biochem Biophys Res Commun 1999, 264:781-788.
14. Habeck H, Odenbach J, Walderich B, Maischein HM, Consortium TS, Schulze-Merker S: Analysis of a zebrafish VEGF receptor mutant reveals specific disruption of angiogenesis. Curr Biol 2002, 12:1405-1412.
15. Lawson ND, Mugford JW, Diamond BA, Weinstein BM: Phospholipase C gamma-1 is required downstream of vascular endothelial growth factor during arterial development. Genes Dev 2003, 17(11):1346-51.
16. Tran J, Master Z, Rak J, Dumont DJ, Kerbel RS: A role for survivin in chemoresistance of endothelial cells mediated by VEGF. Proc Natl Acad Sci 2002, 99(7):4349-4354.
17. Leung T, Chen H, Stauffer AM, et al.: Zebrafish G protein (gamma2) is required for VEGF signaling during angiogenesis. Blood in press. 2006 Mar 14
18. Fukuda S, Fullem LM: Regulation of the inhibitor-of-apoptosis family member survivin in normal cord blood and bone marrow CD34+ cells by hematopoietic growth factors: implication of survivin expression in normal hematopoiesis. Blood 2001, 98(7):2091-2100.
19. Fukuda S, Foster RG, Porter SB, Fullem LM: The antiapoptotic protein survivin is associated with cell cycle entry of normal cord blood CD34+ cells and modulates cell cycle and proliferation of mouse hematopoietic progenitor cells. Blood 2002, 100(7):2463-2471.
20. Pasquier DP, Phung AC, Ymilahi-Ouazzani Q, et al.: Survivin increased vascular development during Xenopus ontogenesis. Development 2002, 129:973-982.
21. Childs S, Chen JN, Garrison DM, Fishman MC: Patternning of angiogenesis in the zebrafish embryos. Development 2002, 129:973-982.
22. Force A, Lynch M, Pickett FB, Amores A, Yan YL, Postlethwait J: Preservation of duplicate genes by complementary, degenerative mutations. Genetics 1999, 151:1531-1545.
23. Tu SP, Jiang XH, Lin MC, et al.: Suppression of survivin expression inhibits in vivo tumorigenicity and angiogenesis in gastric cancer. Cancer Research 2003, 63:7724-7732.
24. Tu SP, Cui JT, Liston P, et al.: Gene therapy for colon cancer by adeno-associated viral vector-mediated transfer of survivin Cys84Ala mutant. Gastroenterology 2005, 128(2):361-75.
25. Kwan TT, Liang R, Verfaille CM, et al.: Regulation of primitive hematopoiesis in zebrafish embryos by the death receptor gene. Exp Hematol 2006, 34(1):27-34.
26. Chen E, Hermanson S, Ekker SC: Syndecan-2 is essential for angiogenic sprouting during zebrafish development. Blood 2004, 103(5):1710-19.
27. Ma AC, Liang R, Leung AY: The role of phospholipase C gamma 1 in primitive hematopoiesis during zebrafish development. Exp Hematol 2007 in press.