

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

**4,800**

Open access books available

**122,000**

International authors and editors

**135M**

Downloads

Our authors are among the

**154**

Countries delivered to

**TOP 1%**

most cited scientists

**12.2%**

Contributors from top 500 universities



**WEB OF SCIENCE™**

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.

For more information visit [www.intechopen.com](http://www.intechopen.com)



# Survivin: Identification of Selective Functional Signaling Pathways in Transformed Cells and Identification of a New Splice Variant with Growth Survival Activity

Louis M. Pelus<sup>1</sup> and Seiji Fukuda<sup>2</sup>

<sup>1</sup>*Department of Microbiology and Immunology  
Indiana University School of Medicine, Indianapolis, IN*

<sup>2</sup>*Department of Pediatrics, Shimane University School of Medicine, Shimane*

<sup>1</sup>*USA*

<sup>2</sup>*Japan*

## 1. Introduction

Survivin is a member of the inhibitors of apoptosis (IAP) family of highly conserved proteins implicated in regulation of mitosis, cytokinesis, cell cycle and apoptosis (Altieri, 2003a; Altieri, 2003b; Fukuda & Pelus, 2006). It is expressed during development but down regulated in most adult tissues. However, Survivin is over-expressed in the majority of solid tumors and leukemias, and is usually associated with higher proliferative index, reduced apoptosis, resistance to chemotherapy and increased rate of tumor recurrence, making it an attractive therapeutic target. We have previously shown that Survivin is expressed and growth factor regulated in normal hematopoietic cells and regulates apoptosis and cell cycle entry (Fukuda et al., 2002; Fukuda et al., 2004; Fukuda & Pelus, 2001; Fukuda & Pelus, 2002). Antagonizing Survivin impairs mouse progenitor cell production in vitro (Fukuda et al., 2002; Fukuda et al., 2004) and loss of function upon conditional deletion in vivo leads to bone marrow ablation as a consequence of loss of stem cell function (Leung et al., 2007). While Survivin is tightly regulated in normal hematopoietic cells, deregulated expression is frequently observed in hematologic diseases particularly those characterized by stem cell expansion. Survivin is aberrantly over expressed in acute myeloid leukemia (Adida et al., 2000; Carter et al., 2001) but down regulated in aplastic anemia where hematopoietic stem and progenitor cells are reduced (Badran et al., 2003). It is now clear that Survivin can regulate cell growth under both physiological and pathological conditions. Therefore, identification of differential signaling cascades between normal and abnormal cells downstream of Survivin is required in order to identify cancer cell specific targets without toxicity to normal cells.

## 2. Survivin mediates aberrant proliferation of hematopoietic progenitor cells transformed by ITD-Flt3

Internal tandem duplication (ITD) mutations of the Flt3 tyrosine kinase receptor are found in many patients with acute leukemia, and are an unfavorable prognostic factor (Fukuda et al.,

2009; Gilliland & Griffin, 2002; Levis et al., 2005). We reported that the combination of Flt3 ligand (FL), stem cell factor (SCF) and thrombopoietin (TPO) induces Survivin expression in human CD34<sup>+</sup> cells (Fukuda & Pelus, 2001), suggesting that Survivin lies downstream of Flt3 signaling. We therefore evaluated the effects of constitutive ITD-Flt3 signaling on Survivin expression. Survivin is up regulated by ITD-Flt3 in whole cells, as well as cells in G<sub>0</sub>/G<sub>1</sub> phase of the cell cycle (Figure 1) as determined by intracellular flow cytometric analyses. This suggests that up regulation of Survivin is not a consequence of cell cycle progression. Similarly, Survivin mRNA was up regulated in Ba/F3 cells by ITD-Flt3 compared to wild-type Flt3 following IL3 withdrawal. Up regulation of Survivin by ITD-Flt3 was associated with growth factor independent proliferation of Ba/F3 cells (Fukuda et al., 2009). We next evaluated whether Survivin plays a role in aberrant growth factor-independent primary HPC proliferation upon transformation by ITD-Flt3. Over expression of human wild-type Flt3 in CFU-GM from control Survivin<sup>fx/fx</sup> or Survivin<sup>fx/fx</sup> mice harboring a tamoxifen-inducible Cre recombinase (Cre-ER<sup>TM</sup>) failed to proliferate in vitro, whereas ectopic expression of ITD-Flt3 resulted in significant growth of CFU-GM in the absence of added growth factors. Treatment with 4OH-Tamoxifen to delete Survivin resulted in a significant reduction in the growth factor-independent proliferation of CFU-GM induced by ITD-Flt3 (Figure 2). Identical results were observed in the immunophenotypically defined c-kit<sup>+</sup>, Sca-1<sup>+</sup>, Lineage<sup>negative</sup> (KSL) population of cells enriched for mouse stem and progenitor cells. These findings suggest that Survivin is involved in the growth factor-independent proliferation of HPC induced by ITD-Flt3 and that antagonizing Survivin may be therapeutically beneficial for acute myeloid leukemia (AML) expressing ITD-Flt3.

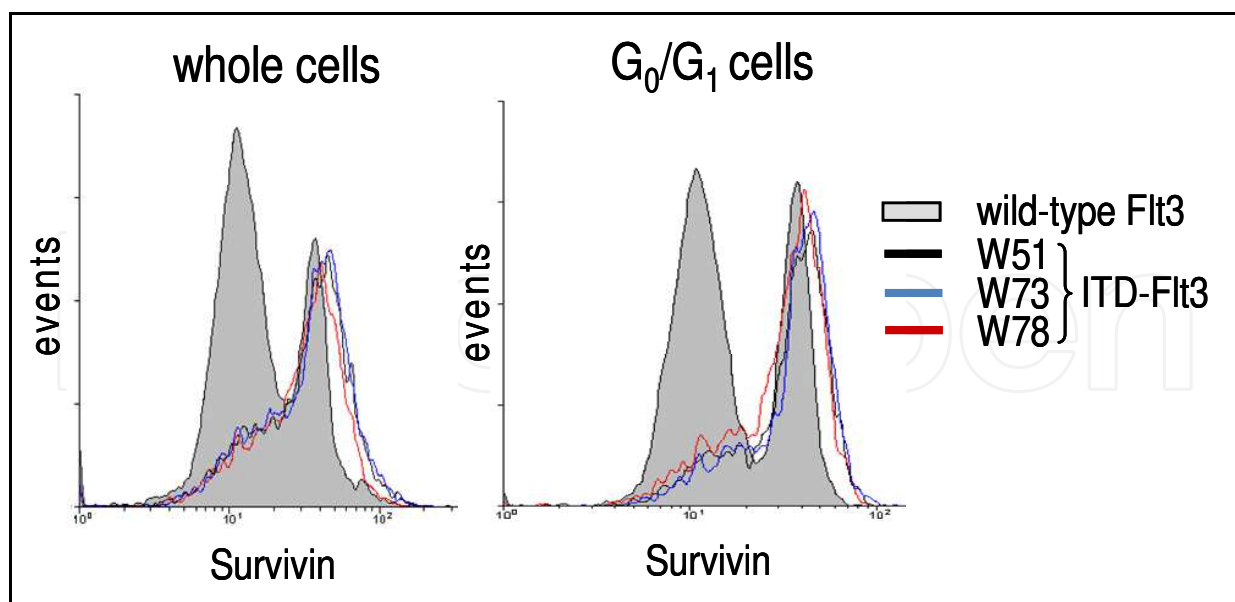


Fig. 1. BaF3 cells expressing wild-type Flt3 or ITD-Flt3 were fixed and stained for Survivin and DNA in whole cells (left) and cells with 2N DNA representing G<sub>0</sub>/G<sub>1</sub> cells (right).

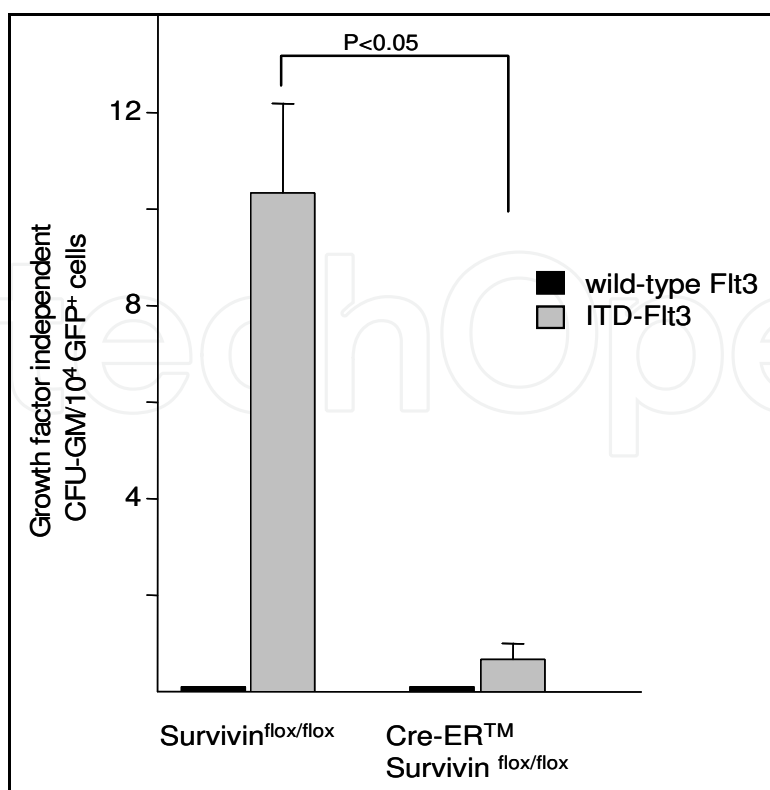


Fig. 2. Wild type Flt3 or ITD-Flt3 were retrovirally transduced into marrow cells from Cre ER Survivin<sup>fx/fx</sup> or control Survivin<sup>fx/fx</sup> mice. Following transduction, cells were plated in a CFU assay in agar with 30% FBS and 1 $\mu$ M 4OH tamoxifen in the absence of hematopoietic growth factors for 2 weeks. Treatment with 4OH-TM significantly reduced growth factor independent CFU-GM induced by ITD-Flt3 in Cre-ERTM Survivin<sup>fx/fx</sup> marrow cells.

### 3. Survivin regulated genes

While it is clear that antagonizing Survivin may reduce the aberrant proliferation of hematopoietic progenitor cells transformed by ITD-Flt3 and that ITD-Flt3 mutations are present in human leukemic stem cells (LSC) (Levis et al., 2005), studies from our group and others indicate that Survivin is a normal regulator of hematopoietic stem and progenitor cells (HSPC) (Fukuda et al., 2002; Fukuda et al., 2004; Leung et al., 2007). This suggests that Survivin targeted therapies will likely result in hematopoietic toxicity. Therefore, identification of differential signaling cascades downstream of Survivin between normal HSPC and cancer stem cells (CSC) or LSC are required to pinpoint targets that can effectively eradicate CSC/LSC with acceptable toxicity to normal HSPC. In order to identify Survivin regulated genes associated with ITD-Flt3 signaling but not normal Flt3 signaling, we evaluated human ITD-Flt3 transformed KSL cells from conditional Survivin knockout mice as a model of normal and leukemic stem cells. We identified 1096 transcripts differentially regulated by Survivin in ITD-Flt3 transformed stem cells [Tables of Survivin regulated genes can be found in (Fukuda et al., 2011)]. Classification of these genes based upon biological process and molecular function defined by Gene Ontology Term using David 2008 showed significant regulation of biological processes, notably phosphate metabolic processes, cell cycle, cell division response to DNA stimulus, RNA biosynthetic processes and transcription. When evaluated based upon molecular function, iron binding,

nucleotide binding, DNA binding and protein kinase activity were the functions most significantly affected by Survivin deletion. Although Survivin is known to inhibit caspases 3, 7 and 10 that mediate apoptosis (Altieri, 2003a; Altieri, 2003b; Fukuda & Pelus, 2006), we did not observe significant changes in genes that mediate apoptosis, suggesting that Survivin can regulate ITD-Flt3 transformed KSL cell fate independent of its ability to inhibit caspase activity. However, microarray analysis was performed on lineage marker negative viable cells that express GFP and stem cell markers and survived in culture, perhaps explaining lack of effects on genes involved in apoptotic pathways. The finding that Survivin deletion affected transcription was surprising since there is no direct evidence that Survivin regulates gene transcription. However, this is consistent with the fact that the *Caenorhabditis elegans* Bir1 Survivin homologue regulates transcription, most likely through effects on histone phosphorylation by Aurora kinase (Kostrouchova et al., 2003). Survivin is essential for activation of Aurora kinase that phosphorylates histone H3 (Bolton et al., 2002; Chen et al., 2003), an event required for transcriptional regulation (Cheung et al., 2000; Li et al., 2002; Nowak & Corces, 2000) and cytokinesis (Wei et al., 1999). Furthermore, modulation of Survivin affects transcription in cancer cells (Asanuma et al., 2004; Balkhi et al., 2008; Takizawa et al., 2007) and transgenic Survivin expression alters gene expression in bladder cells (Salz et al., 2005). Overexpression of Survivin also leads to phosphorylation of the Sp1 transcription factor (Asanuma et al., 2004).

#### 4. Survivin modulates expression of genes deregulated in human AML LSC

The facts that Survivin is required for the self-renewal of ITD-Flt3 transformed KSL cells and ITD-Flt3 is present in leukemic stem cells (LSC) suggests that Survivin is important for LSC fate decisions. When the 1,096 differentially expressed genes in Survivin deleted ITD-Flt3<sup>+</sup> KSL cells was compared with the deregulated gene expression database for human AML stem cells (Majeti et al., 2009), 137 genes were identified in common. Survivin deletion resulted in down-regulation of 79 genes, while 58 genes were up-regulated, implying that these genes are conversely increased and decreased by the presence of Survivin. In contrast, 92 genes were upregulated and 45 genes were downregulated in LSC. Among the 79 genes downregulated by Survivin deletion, 55 were upregulated while 24 genes were downregulated in LSC. Among the 58 transcripts upregulated by Survivin deletion, 21 were downregulated and 37 upregulated in LSC.

Classification of these genes into functional annotation groups using DAVID program identified significantly ( $p < 0.05$ ) enriched functional groups annotated as phosphoprotein, nucleus, acetylation, cell cycle, ATP binding, regulation of EGF signaling pathway, and cell adhesion, with a number of genes showing shared function, suggesting enrichment of gene groups with redundant function. Among the molecules most frequently shared within the functional groups included BGLAP [bone gamma carboxyglutamate protein 1( $\Delta$  -10.7)], Chrac1 [chromatin accessibility complex 1 ( $\Delta$  -4.3)], Hmgb1 [high mobility group box 1 ( $\Delta$  -3.4)] and Smarce1 [SWI ( $\Delta$  -2.9)]. Phosphoprotein, acetylation, DNA binding, ATP binding and DNA replication were significantly enriched when the 76 genes upregulated or downregulated both by Survivin deletion and LSC were classified (Figure 3).

Comparison of the 1,096 differentially Survivin-regulated genes against the mouse HSC database (Ivanova et al., 2002) identified 94 differentially expressed genes. This included 45 genes regulated by Survivin in ITD-Flt3<sup>+</sup> HSC whose expression was selectively enriched in HSC compared to other populations. Notably, Crem, Emp1, Hmga2, Lrrn1, Maff, Myef2,

Rps4x and Sos1 are known to be deregulated in LSC. Comparison of the human LSC associated 137 genes regulated by Survivin in ITD-Flt3<sup>+</sup>KSL cells with differentially regulated genes in normal CD34<sup>neg</sup> KSL cells from control and Survivin deleted mice indicated that coincident with reduction of Survivin expression (10-fold in CD34<sup>neg</sup> KSL cells from CreER<sup>TM</sup>-Survivin <sup>fx/fx</sup> mice compared to control Survivin <sup>fx/fx</sup> in two independent experiments) out of 137 genes, Arg2, Med25, Pmaip1, Pola2, Ube3b, Ephb2 and Rab18 were differentially regulated in both ITD-Flt3<sup>+</sup> KSL cells and normal CD34<sup>neg</sup> KSL cells. In contrast, Cenpa, Cpd, Myef2, Nmt2, Taf1b and Tmpo were down regulated in ITD-Flt3<sup>+</sup> KSL cells while they were upregulated in normal CD34<sup>neg</sup> KSL cells. These findings suggest that 124 genes are regulated by Survivin exclusively in ITD-Flt3<sup>+</sup> KSL cells but not in normal CD34<sup>neg</sup> KSL cells (The complete list of the genes regulated by Survivin in CD34<sup>neg</sup> KSL cells will be reported elsewhere). However, these data clearly indicate that Survivin contributes to deregulation of gene expression in AML stem cells via effects on selective signaling pathways that are distinct from normal HSC that can be potentially targeted for more selective anti-Survivin anti-cancer therapies.

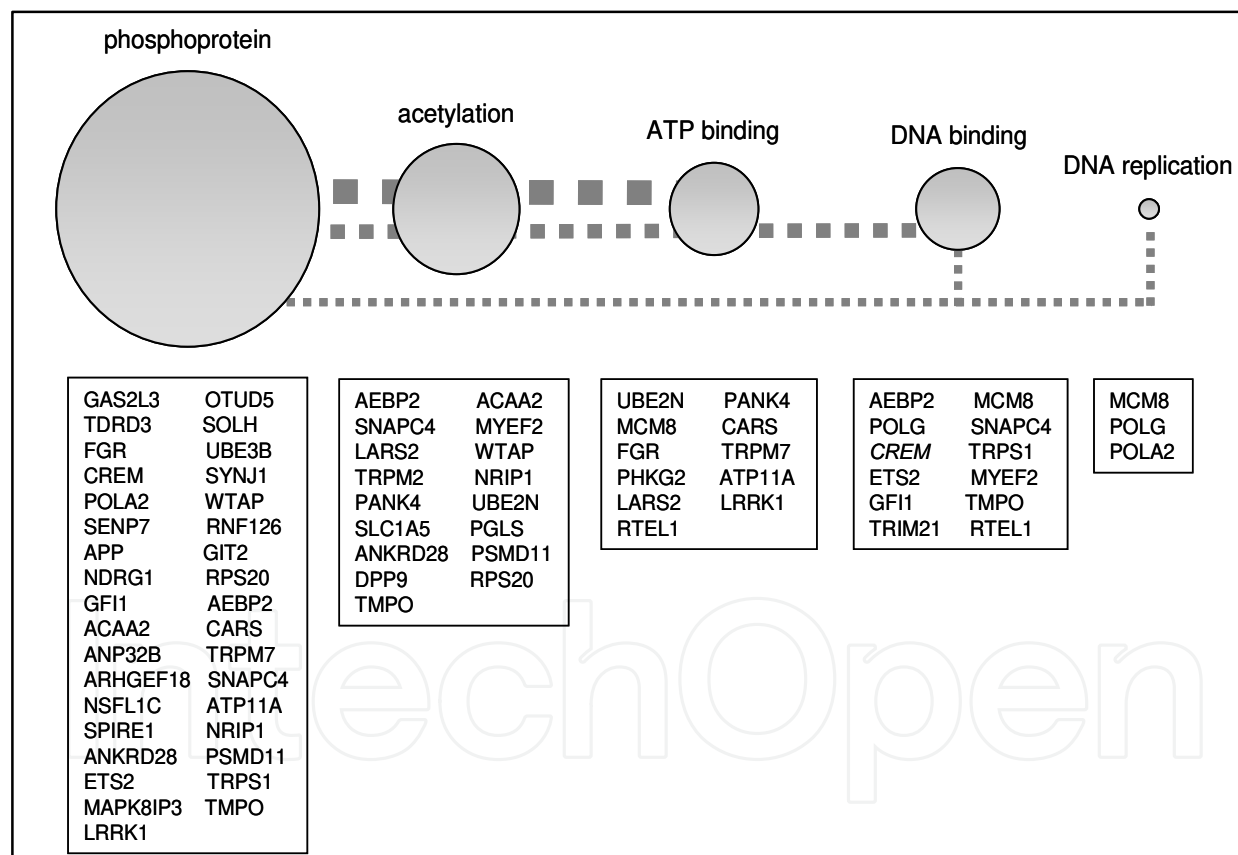


Fig. 3. Genes regulated in common by Survivin and LSC. The 76 genes up-regulated or down regulated by Survivin and LSC were functionally classified by DAVID software. The size of each circle represents the number of genes involved in each functional categories and the thickness of the line indicates the number of genes shared with any function. Functional classification was performed based on Swissprot Keywords. Genes annotated in each functional category are shown in the text box.

## 5. Survivin modulates gene expression in LSC that connects through an EGFR signaling pathway

Functional annotation analysis indicates that genes related with dorso-ventral axis formation or epidermal growth factor receptor signaling pathway (EGFR) are significantly enriched in the shared genes associated with LSC and Survivin signaling in the KEGG database (<http://www.genome.jp/kegg/>) ( $P < 0.03$ ). Similarly, genes associated with regulation of EGFR signaling pathway are enriched in the Gene Ontology database ( $P < 0.02$ ). Our analysis shows that molecules shared by LSC and Survivin signaling are mapped on a functional signaling network that connects through the EGFR pathway (Figure 4). EGFR signaling

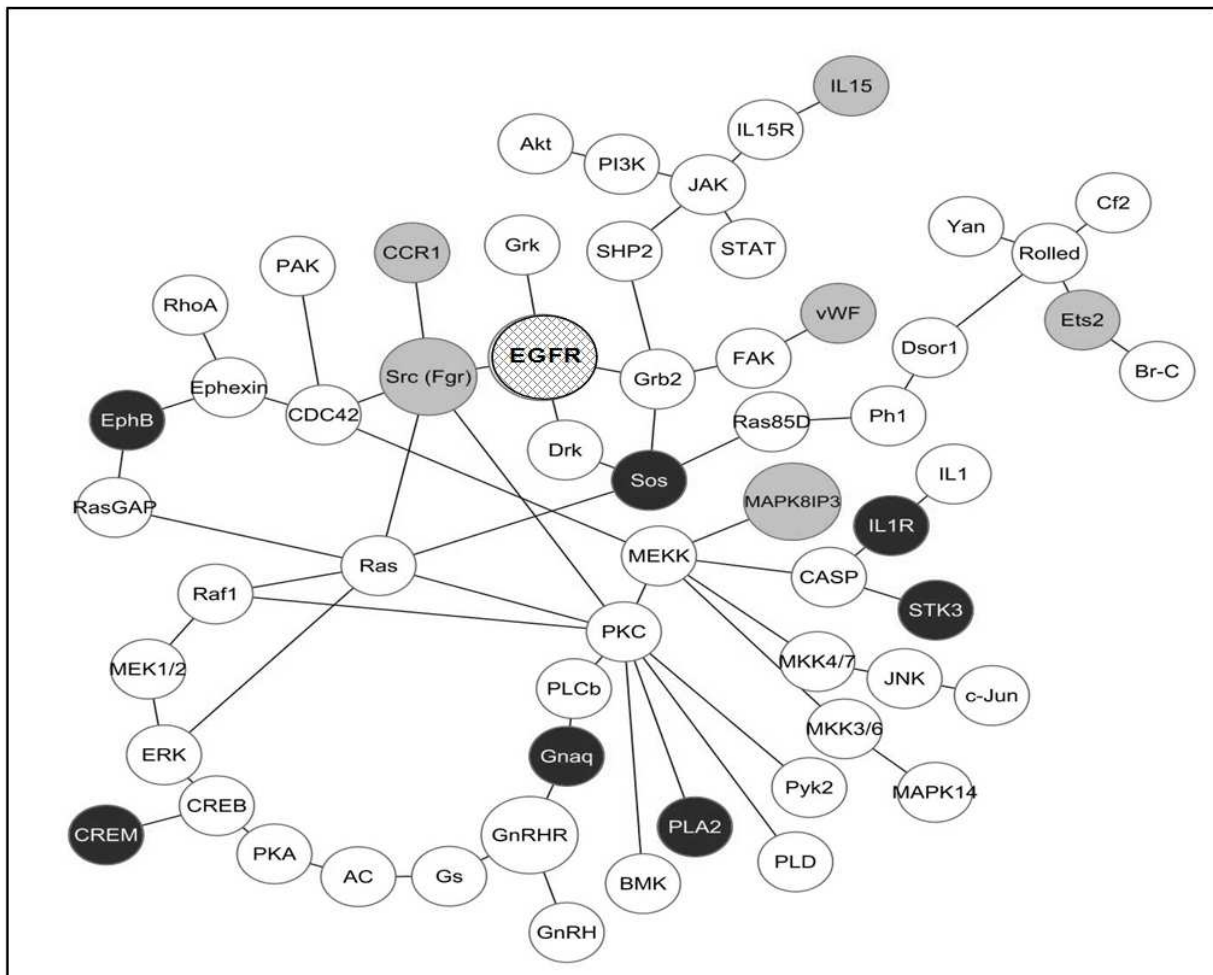


Fig. 4. Genes regulated by Survivin in ITD-Flt3<sup>+</sup> KSL cells and deregulated in LSC are mapped on a functional signaling network associated with the epidermal growth factor receptor signaling pathway. Epidermal growth factor receptor signaling pathway (EGFR) is significantly enriched in the shared genes associated with LSC and Survivin signaling by pathway enrichment analysis in KEGG and Gene Ontology databases. Our analysis shows that 13 molecules shared by LSC and Survivin signaling are mapped on a functional signaling network associated with EGFR. The network was created based on the KEGG database using Cytoscape software. Gray and black circle represents down regulation and up regulation by Survivin gene deletion, respectively. The hatched circle shows EGFR.

activates signaling cascades involved in cell proliferation, such as Src, Sos and MAP-kinases and is known to be dysregulated in a number of solid tumors (Alvarez et al., 2010; Harris & McCormick, 2010). These findings suggest a potential role of EGFR signaling downstream of Survivin in AML stem cells, despite the fact that EGFR is not usually up regulated in AML cells (Hahn et al., 2009). However, recent studies indicate an anti-neoplastic effect of an EGFR inhibitor in AML via off-target effects (Boehrer et al., 2008; Hahn et al., 2009). Thus, our data would support EGFR signaling as a candidate pathway for treatment of patients with AML, even though the number of molecules detected that associates with EGFR signaling is small.

## 6. Survivin splice variants

Survivin, like many genes, is alternatively spliced and variants having both similar or opposite activities have been identified, some of which are of prognostic significance (Fukuda & Pelus, 2006; Sampath & Pelus, 2007). Spliced variants identified to date include Survivin 2 $\alpha$  (Caldas et al., 2005; Mahotka et al., 2002), 2B,  $\Delta$ Ex3 (Ling et al., 2005; Mahotka et al., 1999; Mahotka et al., 2002; Song & Wu, 2005) and 3B (Badran et al., 2004), and some of these variants have been evaluated for their anti- or pro-apoptotic function and ability to interact with wild-type Survivin and other chromosomal passenger proteins (CPC) in some model systems. More recently, several new Survivin splice variants were found in the expressed sequence tag (est) database or described based on RT-PCR using RNA from Molt-4 cells (Li & Ling, 2006; Mola et al., 2007), however the biological functions of these new variants are not known. Overall, with most all the variants reported to date, there is still a considerable lack of understanding of their biology and their function and prognostic value is only now being realized (Sampath & Pelus, 2007). In order to better understand the biology of human Survivin splice variants, we cloned each variant and evaluated their antiapoptotic functions. In the process, we identified a new splice variant we termed Survivin 3 $\gamma$  that is similar to Survivin 3B (Figure 5). To determine if this was truly a novel variant, a forward primer specific for all splice variants and a splice variant specific reverse primer were designed and used to amplify cDNA using a polydT reverse transcribed cDNA library from HL60, K562 and Mo7e-Bcr-abl cells, the products resolved on an agarose gel and ~400bp bands excised, cloned and sequenced. Sequence analysis confirmed the presence of the novel splice variant. Survivin 3 $\gamma$  contains 12 new amino acids at the C-terminus compared to wild-type Survivin and differs from all of the previously reported Survivin splice variants. Secondary structure prediction using Jpred suggests that Survivin 3 $\gamma$  is structurally similar to wild-type Survivin, with the new amino acids predicted to be capable of forming a C-terminal helix that could potentially form the coiled coil domain, even though the length of this helix would be smaller than wild-type Survivin.

## 7. Expression of Survivin 3 $\gamma$

Survivin 3 $\gamma$  expression in primary cancer tissues was determined by quantitative RT-PCR on primary breast and prostate tumor samples obtained in an array format. In contrast to most cancers where wild-type Survivin is the predominant transcript, Survivin 3 $\gamma$  was expressed at higher levels in benign prostate tissue and at equivalent levels in breast cancer cells. In primary breast cancer samples a statistically significant increase in wild-type Survivin expression at stages 1, 2 and 3 compared to control normal breast tissue was observed, with



no significant difference observed between wild-type Survivin expression at stage 1 compared to later stages. In 7 normal breast tissue samples wild-type Survivin and Survivin 3 $\gamma$  were expressed at the same level. However, in contrast to expression of wild-type Survivin, Survivin 3 $\gamma$  expression remained relatively constant at all stages of disease, resulting in statistically significant ( $p < 0.005$ ) lower relative expression to wild-type Survivin.

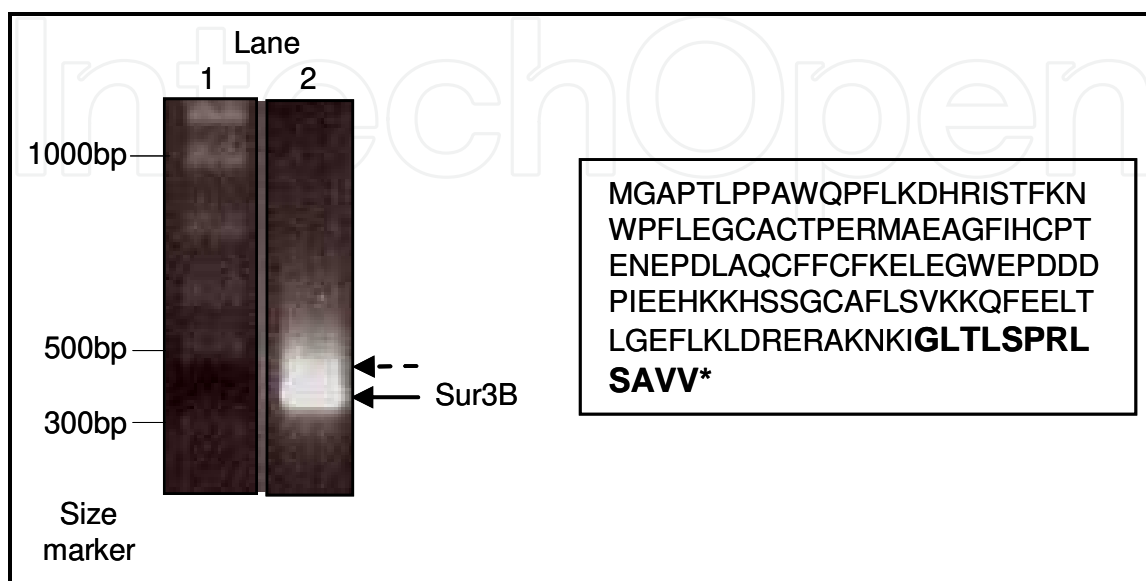


Fig. 5. Agarose gel electrophoresis of cDNA amplified from HL60 cells (lane 2). The Survivin-3B variant is indicated by a solid arrow, the dotted arrow points to the slower migrating band. Amino acid sequence of Survivin-3 $\gamma$  is shown on the right. Bold letters indicate novel sequence distinct from wild-type Survivin. The stop codon is indicated by the \*.

In 48 prostate cancers at different stages of the disease, wild-type Survivin expression was higher in stage 1 prostate cancer compared to benign hyperplasia; however sample size was too small to evaluate statistical significance. No clear over-expression of wild-type Survivin was seen at higher stages, except for a single stage 4 sample. The expression of Survivin 3 $\gamma$  was higher than wild-type Survivin in benign hyperplasia tissue and comparison of the relative expression of wild-type Survivin and Survivin 3 $\gamma$  at each stage of prostate cancer indicated that Survivin 3 $\gamma$  transcripts remained higher than wild-type Survivin at all stages except stage 1. To our knowledge, the higher expression of Survivin 3 $\gamma$  in benign prostate hyperplasia is the first demonstration of a Survivin splice variant expressed at higher levels than wild-type Survivin and suggest that it may play a role in normal cell function.

The finding that Survivin 3 $\gamma$  expression was higher than wild-type Survivin in benign prostate hyperplasia (BPH) samples and did not change with stage of disease is consistent with one study (Kaur et al., 2004) where Survivin expression was detected by immunohistochemistry. However, other reports show increased Survivin with stage of disease either at the RNA level by standard RT-PCR and gel electrophoresis (Kishi et al., 2004; Krajewska et al., 2003) or by immunohistochemistry (Krajewska et al., 2003). The difference between our study and those reported using standard RT-PCR could be due to differences in sensitivity of the method used. SYBR green quantitative RT-PCR is more sensitive and efficient in amplifying smaller amplicons than conventional RT-PCR. We also show that expression of wild-type Survivin and Survivin 3 $\gamma$  were comparable in normal

breast tissues and that the expression of Survivin increased at stage 1 and remained high at all stages, whereas a recent report (Sohn et al., 2006) showed Survivin expression increased gradually at all disease stages. This difference could again be due to different methodologies and sensitivity between RNA analyses versus immunohistochemistry. Since the Survivin 3 $\gamma$  splice variant was identified in the human HL-60 promyelocytic cell line, we evaluated its expression in other hematopoietic cells by quantitative RT-PCR using SYBR Green. While wild-type Survivin was the predominant transcript identified in all cell lines tested, Survivin 3 $\gamma$  transcripts represented ~0.7-6% of the total Survivin transcripts detected. The absolute expression of Survivin 3 $\gamma$  was lowest in growth factor dependant cells and highest in growth factor independent cells. Analysis of Survivin 3 $\gamma$  in primary umbilical cord blood (UCB) CD34<sup>+</sup> cells that are enriched for hematopoietic stem and progenitor cells showed that Survivin 3 $\gamma$  was expressed at significantly higher levels than wild-type Survivin. This is in contrast to most splice variants that are expressed at lower levels than wild-type Survivin in these cells. However, growth factor stimulation induced a dramatic increase in expression of wild-type Survivin after 48 hours consistent with our previous findings (Fukuda et al., 2002; Fukuda & Pelus, 2001; Fukuda & Pelus, 2002), whereas the expression of Survivin 3 $\gamma$  increased only slightly, therefore relative levels compared to wild-type Survivin decreased by ~2-fold. This suggests perhaps that Survivin 3 $\gamma$  may play a role in hematopoietic stem cell maintenance.

### 8. Survivin 3 $\gamma$ demonstrates antiapoptotic activity

To evaluate the function of the novel Survivin 3 $\gamma$  splice variants, we stably expressed human (Hu) wild-type Survivin with or without an N-terminal Flag-tag or Survivin 3 $\gamma$  with an N-terminal HA-tag in murine YAC-1 lymphoma cells. Wild-type HuSurvivin, wild-type Flag-HuSurvivin and HA-Survivin 3 $\gamma$  were all expressed, although their levels of expression varied, with wild-type HuSurvivin expressing at 31-fold, whereas wild-type Flag-HuSurvivin and HA-Survivin 3 $\gamma$  were expressed at 76- and 63-fold higher levels, respectively than the empty vector control cells at the RNA level. Western blot analysis using a rabbit anti-Survivin polyclonal antibody that recognizes both the human and the murine Survivin showed background levels of murine Survivin as well as the presence of each of the transduced Survivins, indicating that each of the splice variants was translated. Analysis of band intensity indicated that wild-type HuSurvivin was expressed at the highest level followed by wild-type Flag-HuSurvivin with HA-Survivin 3 $\gamma$  expressed at a lower level. The same pattern of expression was observed with a mouse anti-Survivin monoclonal antibody that recognizes predominantly human Survivin.

As a measure of anti-apoptotic function we evaluated resistance of YAC-1 cells transduced with wild-type Survivin, wild-type Flag-Survivin and HA-Survivin 3 $\gamma$  to the chemotherapeutic drugs Etoposide and Taxol at their optimal therapeutic dose. Treatment of cells with Taxol showed that, as expected, over expression of either wild-type Survivin or wild-type Flag-Survivin provided 2.7- and 2.8-fold resistance to Taxol-induced cell death, respectively, compared to YAC-1 cells transduced with vector control (Figure 6). Ectopic expression of HA-Survivin 3 $\gamma$  provided 2.6-fold resistance. In multiple experiments, the IC<sub>50</sub>'s for wild-type HuSurvivin, wild-type Flag-HuSurvivin and HA-Survivin 3 $\gamma$  were significantly higher than control ( $2.85 \pm 0.32$ ;  $P < 0.05$ ). YAC-1 cells expressing wild-type

Survivin also showed significant resistance to Etoposide. Wild-type Flag-Survivin and HA-Survivin 3 $\gamma$  conferred resistance to Etoposide in YAC-1 cells to a similar degree as wild-type Survivin. These studies suggest that expression of Survivin 3 $\gamma$  could potentially contribute to drug resistance. Expression levels of murine wild-type Survivin and the only known murine Survivin splice variant, Survivin-121 (Conway et al., 2000) were unchanged (not shown), indicating that changes in endogenous murine Survivin levels were not involved in the increase in resistance to chemotherapeutic compounds. These findings suggest that wild-type and Survivin 3 $\gamma$  proteins possess equivalent anti-apoptotic function. Since crystal structure of wild-type Survivin indicates that the C-terminal helix could be involved in protein-protein interaction (Chantalat et al., 2000) and required for interaction with the chromosomal passenger protein, INCEP (Wheatley et al., 2004), whereas the N-terminus of wild-type Survivin was unordered (Chantalat et al., 2000), we hypothesized that addition of a C-terminal tag would hinder protein-protein interactions, therefore we engineered N-terminal tags into all the Survivin cDNAs. The presence of HA-tag at the N-terminus of the Survivin coding region did not affect activity since they were equivalent to N-terminal Flag-tagged wild-type Survivin, which itself behaved similar to wild-type Survivin at least for drug sensitivity. The facts that Survivin 3 $\gamma$  confers drug resistance/anti-apoptotic function to two different classes of chemotherapeutic drugs Taxol, a microtubule destabilizing agent, and Etoposide, a topoisomerase II inhibitor, as well as growth factor independence similar to wild-type Survivin, and that it may be preferentially associated with maintenance of stem cell quiescence, makes it an interesting potential therapeutic target .

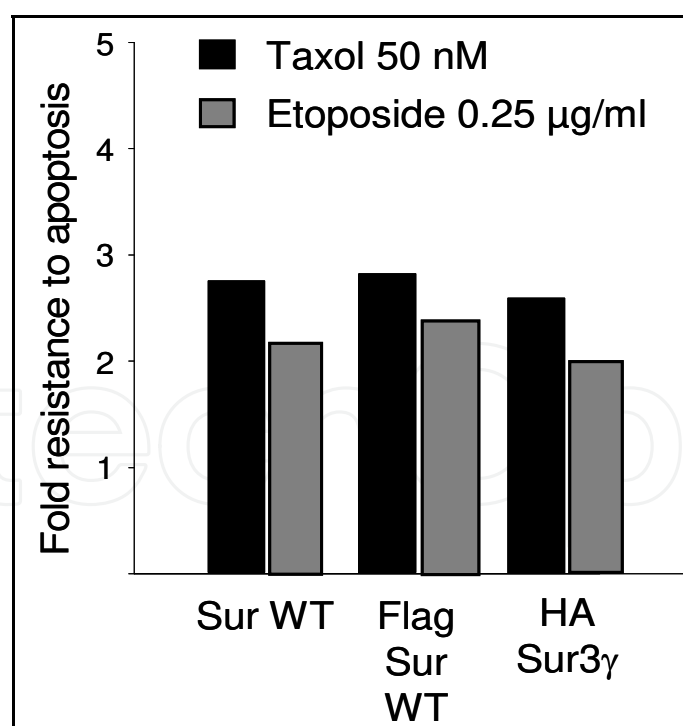


Fig. 6. Response of YAC1 cells over expressing wild-type human Survivin (SurWT), wild-type human Flag-Survivin (Flag-Sur WT) and HA Survivin 3 $\gamma$  (HA-Sur3 $\gamma$ ) to chemotherapeutic drugs. Fold resistance of stable transduced YAC-1 cells treated with 50 nM Taxol or 0.25  $\mu$ g/ml Etoposide.

## 9. Summary

We have shown that ITD-Flt3 mutations increase expression of Survivin, which regulates development of ITD-Flt3<sup>+</sup> acute leukemia, clearly indicating a potential therapeutic benefit for antagonizing Survivin in patients with ITD-Flt3<sup>+</sup> AML (Fukuda et al., 2009; Fukuda et al., 2011; Sampath & Pelus, 2007). However, we have shown that Survivin also regulates normal HSPC function (Fukuda et al., 2004; Fukuda & Pelus, 2002; Leung et al., 2007; Sampath & Pelus, 2007); raising caution that targeting Survivin can have hematopoietic toxicity. Therefore, identification of differential signaling cascades downstream of Survivin between normal hematopoietic cells versus cancer and leukemia stem cells is required to pinpoint targets that can lead to effective anti-malignant therapies with acceptable normal stem cell toxicity. Our genetic profiling using normal and ITD-Flt3 transformed cells from conditional Survivin knockout mice has identified a panel of genes that are specifically regulated by Survivin in ITD-Flt3 transformed cells and known to be deregulated in human LSC that can be potentially targeted for selective anti-leukemia therapy.

Inherited and acquired changes in pre-mRNA splicing have been documented to play a significant role in human disease development and loss of fidelity or variation of the splicing process may play a major role in carcinogenesis (Fackenthal & Godley, 2008; Matlin et al., 2005; Skotheim & Nees, 2007). Splice variants that are found predominantly in tumors have clear diagnostic value (Brinkman, 2004; Caballero et al., 2001) and may provide potential drug targets. Splice variants of Survivin have been reported but their prognostic value is not clear, partially based upon detection methods that are not sufficiently specific (Sampath & Pelus, 2007), or due to lack of information on biological function. We have identified a novel Survivin splice variant with anti-apoptotic activity similar to wild-type Survivin, at least with respect to sensitivity to chemotherapeutic drugs. We characterized its expression in several transformed cell lines, and similar to other Survivin splice variants, wild-type Survivin is the predominant transcript. However, in contrast to wild-type Survivin, Survivin 3 $\gamma$  expression does not change with increasing stage/severity of primary prostate and breast cancers. In addition, unlike other Survivin splice variants, Survivin 3 $\gamma$  is expressed at higher levels than wild-type Survivin in primary HSPC, and their lack of increase with cell proliferation suggests that they may play a role in stem cell maintenance. Since Survivin 3 $\gamma$  was cloned from and found to be highly expressed in hematopoietic cells, it will be particularly interesting to evaluate its expression in hematopoietic malignancies and their correlation with disease/stage/survival. Furthermore, since ITD-Flt3 mutations increase Survivin expression, it will be interesting to determine if they also modulate Survivin 3 $\gamma$  expression and how this relates to disease progression and prognostic value. Given the specific genes regulated by Survivin in leukemic stem cells, it will also be of interest whether Survivin 3 $\gamma$  elicits overlapping or divergent gene products that can be utilized for development of anti-leukemia therapies.

## 10. References

- Adida, C. et.al (2000). Expression and prognostic significance of survivin in de novo acute myeloid leukaemia. *Br J Haematol*, Vol.111:196-203.
- Altieri, D.C. (2003a). Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene*, Vol.22:8581-8589.

- Altieri, D.C. (2003b). Validating survivin as a cancer therapeutic target. *Nat Rev Cancer*, Vol.3:46-54.
- Alvarez, R.H.; Valero, V. & Hortobagyi, G.N. (2010). Emerging targeted therapies for breast cancer. *J Clin Oncol*, Vol.28:3366-3379.
- Asanuma, K. et.al (2004). Survivin enhances Fas ligand expression via up-regulation of specificity protein 1-mediated gene transcription in colon cancer cells. *J Immunol*, Vol.172:3922-3929.
- Badran, A. et.al (2004). Identification of a novel splice variant of the human anti-apoptosis gene survivin. *Biochem Biophys Res Commun*, Vol.314:902-907.
- Badran, A. et.al (2003). Expression of the anti-apoptotic gene survivin in myelodysplastic syndrome. *Int J Oncol*, Vol.22:59-64.
- Balkhi, M.Y. et.al (2008). AML1/ETO-induced survivin expression inhibits transcriptional regulation of myeloid differentiation. *Exp Hematol*, Vol.36:1449-1460.
- Boehrer, S. et.al (2008). Erlotinib exhibits antineoplastic off-target effects in AML and MDS: a preclinical study. *Blood*, Vol.111:2170-2180.
- Bolton, M.A. et.al (2002). Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation. *Mol Biol Cell*, Vol.13:3064-3077.
- Brinkman, B.M. (2004). Splice variants as cancer biomarkers. *Clin Biochem*, Vol.37:584-594.
- Caballero, O.L. et.al (2001). Alternative spliced transcripts as cancer markers. *Dis Markers*, Vol.17:67-75.
- Caldas, H.; Honsey, L.E. & Altura, R.A. (2005). Survivin 2alpha: a novel Survivin splice variant expressed in human malignancies. *Mol Cancer*, Vol.4:11-
- Carter, B.Z. et.al (2001). Cytokine-regulated expression of survivin in myeloid leukemia. *Blood*, Vol.97:2784-2790.
- Chantalat, L. et.al (2000). Crystal structure of human survivin reveals a bow tie-shaped dimer with two unusual alpha-helical extensions. *Mol Cell*, Vol.6:183-189.
- Chen, J. et.al (2003). Survivin enhances Aurora-B kinase activity and localizes Aurora-B in human cells. *J Biol Chem*, Vol.278:486-490.
- Cheung, P. et.al (2000). Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol Cell*, Vol.5:905-915.
- Conway, E.M. et.al (2000). Three differentially expressed survivin cDNA variants encode proteins with distinct antiapoptotic functions. *Blood*, Vol.95:1435-1442.
- Fackenthal, J.D. Godley, L.A. (2008). Aberrant RNA splicing and its functional consequences in cancer cells. *Dis Model Mech*, Vol.1:37-42.
- Fukuda, S. et.al (2011). Survivin selectively modulates genes deregulated in human leukemia stem cells. *J Oncol*, Vol.2011:doi:10.1155/2011/94936-
- Fukuda, S. et.al (2002). The antiapoptosis 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*, Vol.100:2463-2471.
- Fukuda, S.; Mantel, C.R. & Pelus, L.M. (2004). Survivin regulates hematopoietic progenitor cell proliferation through p21WAF1/Cip1-dependent and -independent pathways. *Blood*, Vol.103:120-127.
- Fukuda, S. Pelus, L.M. (2001). 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*, Vol.98:2091-2100.
- Fukuda, S. Pelus, L.M. (2002). Elevation of Survivin levels by hematopoietic growth factors occurs in quiescent CD34+ hematopoietic stem and progenitor cells before cell cycle entry. *Cell Cycle*, Vol.1:322-326.
- Fukuda, S. Pelus, L.M. (2006). Survivin, a cancer target with an emerging role in normal adult tissues. *Mol Cancer Ther*, Vol.5:1087-1098.
- Fukuda, S. et.al (2009). Survivin mediates aberrant hematopoietic progenitor cell proliferation and acute leukemia in mice induced by internal tandem duplication of Flt3. *Blood*, Vol.114:394-403.
- Gilliland, D.G. Griffin, J.D. (2002). The roles of FLT3 in hematopoiesis and leukemia. *Blood*, Vol.100:1532-1542.
- Hahn, C.K. et.al (2009). Proteomic and genetic approaches identify Syk as an AML target. *Cancer Cell*, Vol.16:281-294.
- Harris, T.J. McCormick, F. (2010). The molecular pathology of cancer. *Nat Rev Clin Oncol*, Vol.7:251-265.
- Ivanova, N.B. et.al (2002). A stem cell molecular signature. *Science*, Vol.298:601-604.
- Kaur, P. et.al (2004). Survivin and Bcl-2 expression in prostatic adenocarcinomas. *Arch Pathol Lab Med*, Vol.128:39-43.
- Kishi, H. et.al (2004). Expression of the survivin gene in prostate cancer: correlation with clinicopathological characteristics, proliferative activity and apoptosis. *J Urol*, Vol.171:1855-1860.
- Kostrouchova, M. et.al (2003). BIR-1, a Caenorhabditis elegans homologue of Survivin, regulates transcription and development. *Proc Natl Acad Sci U S A*, Vol.100:5240-5245.
- Krajewska, M. et.al (2003). Elevated expression of inhibitor of apoptosis proteins in prostate cancer. *Clin Cancer Res*, Vol.9:4914-4925.
- Leung, C.G. et.al (2007). Requirements for survivin in terminal differentiation of erythroid cells and maintenance of hematopoietic stem and progenitor cells. *J Exp Med*, Vol.204:1603-1611.
- Levis, M. et.al (2005). Internal tandem duplications of the FLT3 gene are present in leukemia stem cells. *Blood*, Vol.106:673-680.
- Li, F. Ling, X. (2006). Survivin study: an update of "what is the next wave"? *J Cell Physiol*, Vol.208:476-486.
- Li, J. et.al (2002). Involvement of histone methylation and phosphorylation in regulation of transcription by thyroid hormone receptor. *Mol Cell Biol*, Vol.22:5688-5697.
- Ling, X. et.al (2005). Differential expression of survivin-2B and survivin-DeltaEx3 is inversely associated with disease relapse and patient survival in non-small-cell lung cancer (NSCLC). *Lung Cancer*, Vol.49:353-361.
- Mahotka, C. et.al (2002). Differential subcellular localization of functionally divergent survivin splice variants. *Cell Death Differ*, Vol.9:1334-1342.
- Mahotka, C. et.al (1999). Survivin-deltaEx3 and survivin-2B: two novel splice variants of the apoptosis inhibitor survivin with different antiapoptotic properties. *Cancer Res*, Vol.59:6097-6102.
- Majeti, R. et.al (2009). Dysregulated gene expression networks in human acute myelogenous leukemia stem cells. *Proc Natl Acad Sci U S A*, Vol.106:3396-3401.

- Matlin, A.J.; Clark, F. & Smith, C.W. (2005). Understanding alternative splicing: towards a cellular code. *Nat Rev Mol Cell Biol*, Vol.6:386-398.
- Mola, G. et.al (2007). Exonization of Alu-generated splice variants in the survivin gene of human and non-human primates. *J Mol Biol*, Vol.366:1055-1063.
- Nowak, S.J. Corces, V.G. (2000). Phosphorylation of histone H3 correlates with transcriptionally active loci. *Genes Dev*, Vol.14:3003-3013.
- Salz, W. et.al (2005). A survivin gene signature predicts aggressive tumor behavior. *Cancer Res*, Vol.65:3531-3534.
- Sampath, J. Pelus, L.M. (2007). Alternative splice variants of survivin as potential targets in cancer. *Curr Drug Discov Technol*, Vol.4:174-191.
- Skotheim, R.I. Nees, M. (2007). Alternative splicing in cancer: noise, functional, or systematic? *Int J Biochem Cell Biol*, Vol.39:1432-1449.
- Sohn, D.M. et.al (2006). Expression of survivin and clinical correlation in patients with breast cancer. *Biomed Pharmacother*, Vol.60:289-292.
- Song, Z. Wu, M. (2005). Identification of a novel nucleolar localization signal and a degradation signal in Survivin-deltaEx3: a potential link between nucleolus and protein degradation. *Oncogene*, Vol.24:2723-2734.
- Takizawa, B.T. et.al (2007). Downregulation of survivin is associated with reductions in TNF receptors' mRNA and protein and alterations in nuclear factor kappa B signaling in urothelial cancer cells. *Cancer Invest*, Vol.25:678-684.
- Wei, Y. et.al (1999). Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. *Cell*, Vol.97:99-109.
- Wheatley, S.P. et.al (2004). Aurora-B phosphorylation in vitro identifies a residue of survivin that is essential for its localization and binding to inner centromere protein (INCENP) in vivo. *J Biol Chem*, Vol.279:5655-5660.

IntechOpen



## **Advances in Cancer Therapy**

Edited by Prof. Hala Gali-Muhtasib

ISBN 978-953-307-703-1

Hard cover, 568 pages

**Publisher** InTech

**Published online** 21, November, 2011

**Published in print edition** November, 2011

The book "Advances in Cancer Therapy" is a new addition to the InTech collection of books and aims at providing scientists and clinicians with a comprehensive overview of the state of current knowledge and latest research findings in the area of cancer therapy. For this purpose research articles, clinical investigations and review papers that are thought to improve the readers' understanding of cancer therapy developments and/or to keep them up to date with the most recent advances in this field have been included in this book. With cancer being one of the most serious diseases of our times, I am confident that this book will meet the patients', physicians' and researchers' needs.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Louis M. Pelus and Seiji Fukuda (2011). Survivin: Identification of Selective Functional Signaling Pathways in Transformed Cells and Identification of a New Splice Variant with Growth Survival Activity, *Advances in Cancer Therapy*, Prof. Hala Gali-Muhtasib (Ed.), ISBN: 978-953-307-703-1, InTech, Available from: <http://www.intechopen.com/books/advances-in-cancer-therapy/survivin-identification-of-selective-functional-signaling-pathways-in-transformed-cells-and-identific>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen