Biomarkers, Genomics, Proteomics, and Gene Regulation

Generation of a Novel Transgenic Mouse Model for Bioluminescent Monitoring of Survivin Gene Activity *in Vivo* at Various Pathophysiological Processes

Survivin Expression Overlaps with Stem Cell Markers

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Survivin has been implicated to play an important role in various pathophysiological processes. However, because of a lack of appropriate animal models, the role and dynamic expression of survivin during pathophysiology are not well defined. We generated a human survivin gene promoter-driven luciferase transgenic mouse model (SPlucTg) so that dynamic survivin gene activity can be monitored during various pathophysiological conditions using *in vivo* imaging. Our results show that, consistent with survivin positivity in testis, luciferase activity in normal SPlucTg mice was detected in the testis of male mice. Furthermore, similar to the known requirement of transient expression of survivin for pathophysiological responses, we observed a transient luciferase expression in castrated SPlucTg male mice after supplement of androgen. Significantly, it was reported that survivin expression turns on during mouse liver injury and regeneration; a transient and dose-dependent luciferase expression in the mouse liver was observed after administration of carbon tetrachloride into SPlucTg mice. We further demonstrated that luciferase activity closely correlates with endogenous survivin expression. We also demonstrated that only a subset of cells expresses survivin, and its expression overlaps with the expression of several stem cell markers tested. Thus, we have generated a unique animal model for analysis of diverse pathophysiological processes and possible stem cell distribution/

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Evidence indicates that survivin, an inhibitor of apoptosis (IAP) family protein and a promoter of cell cycle and mitosis,^{1–3} plays an important role in cancer initiation⁴ and angiogenesis,^{5–7} tumor progression, drug/radiation resistance,⁸ and tumor relapse.^{3,9} Survivin may also play a role in other pathophysiological processes.^{10–12} These include embryonic development, hematopoietic cell survival and proliferation, T-cell development, multiple sclerosis, brain pathology, and pathophysiology of other organs including liver, pancreas, gastrointestinal, testes, endometrium, and placenta.¹¹ However, studies indicated that the behavior of survivin expression, subcellular localization, and/or gene regulation appear to be different during normal tissue turnover or repair versus cancer initiation and progression.¹¹ Furthermore, in some cases, normal cell division, survival, and development happen in the absence of survivin¹³ or with differential requirement of survivin.¹⁴ Additionally, because of increased expression of survivin in cancer, the interaction of survivin molecules with other proteins may differ from normal cells and/or may exclusively occur in cancer

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cells.^{15,16} This partially explains the observations indicating that whereas abrogation of survivin expression or functions triggers significant apoptosis of cancer cells, it has minimal effects on normal cells and tissues.^{17–24} Thus, survivin appears to be an ideal cancer preventive and therapeutic target.

Targeting survivin for cancer treatment might cause little toxicity to normal tissues. Indeed, many natural chemopreventive and chemotherapeutic dietary components, such as resveratrol, silibinin, and sulindac²⁵ as well as selenium,²⁶ retinoid,^{27,28} and vitamin D (eg, calcitriol)²⁹ down-regulate survivin expression in cancer cells. This is consistent with a role for survivin in oncogenesis. However, because of the lack of an appropriate *in vivo* animal model system, the role for survivin either in cancer-related or in other *in vivo* pathological processes¹¹ remains to be elucidated.

Here, we report the generation of a novel transgenic mouse model that expresses the luciferase reporter gene as the human survivin gene surrogate under the control of a human survivin gene promoter. We demonstrated that this new mouse model possesses an active and highly responsive luciferase reporter system, which reflects the dynamic expression of endogenous survivin during pathology. We further show that survivin expression overlaps with several stem cell markers, which suggests that survivin may be a good stem cell marker as well. Our model has several unique features for *in vivo* imaging in comparison with the model previously reported.³⁰ In our model, we used the human survivin promoter with sufficient length (4.5 kb), instead of using a mouse survivin proximal promoter (0.8 kb; as a note, the design in Xia et al's report³⁰ is good for studying the relationship between survivin expression and cell cycle/mitosis). Importantly, using the luciferase reporter as a surrogate to monitor survivin gene activity during various pathophysiological processes will increase sensitivity by several orders of magnitude in comparison with using GFP as a reporter for *in vivo* imaging, although GFP can provide strong signals. Therefore, with our model, it is now possible to monitor endogenous survivin gene activity through *in vivo* imaging of its surrogate luciferase activity during various pathophysiological processes. This mouse model has the potential to delineate the dynamic expression and role of survivin in cancer and other pathophysiological processes.

Materials and Methods

Generation of Human Survivin Promoter-Driven Luciferase-Transgenic (SPLucTg) Mice

A 4.5-kb human survivin promoter sequence starting from –1 at the 3'-end (the A in ATG codon of the survivin gene is designated as +1) that reflects the expression behavior of the endogenous survivin gene (cell cycle-dependent and -independent transcription control)^{31–33} was isolated and inserted upstream of the firefly luciferase reporter gene at the Sall and HindIII sites of the pLuc vector, which is a home-made vector using pGEM3 as

Figure 1. Generation of the human survivin promoter-driven luciferase reporter-transgenic (SPLucTg) mice. **A:** Diagram of the structure of the transgene cassette. Four primers used in PCR to determine genotype of the transgenic mouse are shown. **B:** Representative genotyping results for the transgenic mice. **C:** The luciferase expression profile in genotype-positive SPLucTg mice was determined by *in vivo* imaging. Red arrows indicate tests that show luciferase activity. ♂ indicates male mice; ♀, female mice.

the backbone. The resulting vector was first tested in both cancer (HeLa, MCF-7, PC-3, A549, and E0771) and HEK293 cells for testing activity. The verified vector was then digested with Sall and SacI and the transgene cassette was purified using cesium chloride; the bacterial plasmid backbone was discarded. The purified transgene cassette DNA was microinjected into the pronuclei of day –0.5 embryos extracted from C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME). Injected embryos were implanted into pseudo-pregnant female Swiss Webster mice (Taconi, Hudson, NY). Tail biopsies and subsequent DNA isolation was performed when the resulting pups were three to four weeks of age for genotype analysis. Presence of the transgene cassette was identified by PCR amplification of both the human survivin gene promoter and the luciferase gene sequence as shown in Figure 1A. Two human survivin promoter-specific primers at the 5'-region of the promoter (that do not recognize the endogenous mouse survivin sequence), P1 (5'-GCAG-GAGAATCGCTTGAACCCGTGG-3') and P2 (5'-CCACT-CACTTCTCTGGTTCTATGGCC-3'), were used to amplify a 308-bp PCR product. Two firefly luciferase gene-specific primers at the 3'-region of the gene, P3 (5'-GTG-GATTACGTGCGCCAGTCAAGTAAC-3') and P4 (5'-CAATAGCTAAGAATTTTCGTATCGCTG-3'), were used

to amplify a 200-bp PCR product. The PCR reaction and condition are as follows: 200 nmol/L for each primer, 1×PCR buffer with 1.2 mmol/L Mg^{2+} , 200 μ mol/L for each dNTP, 0.5% DMSO, 0.5 to 1 unit TaqDNA polymerase (usb, Cleveland, OH), and 1 to 2 μ l from total 150 tail DNA as templates in 25 μ l total volume. PCR amplification was performed for 40 cycles at 94°C × 4 minutes for pre-denature and then 40 cycle of 94°C × 30 seconds, 54°C × 30 seconds and 72°C × 1 minute, followed by 94°C × 10 minutes and then held at 4°C. Of note, plasmid DNA containing the survivin promoter-luciferase cassette was used as positive control. Of a further note, the procedure for generation of transgenic mice above was covered by the RPCI IACUC-approved mouse protocol 912M. Other mouse procedures (see below) were covered by 1070M and 1072M mouse protocols.

Mouse Whole Body *in Vivo* Imaging

Animal whole body imaging was performed to detect luciferase reporter activity using the Xenogen IVIS® *in vivo* Imaging System (Caliper Life Science, Hopkinton, MA). In each measurement, SPLucTg mice were intraperitoneally injected with D-luciferin potassium salt dissolved in PBS at a dose of 75 mg/kg. Ten minutes after D-luciferin injection, mice were anesthetized with isoflurane at 5% mixed with air in a separate induction chamber for one minute and then transferred into the *in vivo* imaging chamber for detection of luciferase activity for two minutes at a maintenance condition of anesthesia at 2% to 2.5% of isoflurane.

Mouse Castration and Tissue Re-Proliferation

Twelve-week-old SPLucTg mice with a C57BL/6 background were castrated as described previously.³⁴ After two weeks (14 days) the castrated mice were subcutaneously implanted with testosterone pellets. Luciferase activity was monitored using *in vivo* imaging on day 1, 3, 5, 7, 9, 11, 14, and 17, post androgen replacement.

Liver Injury and Regeneration

For the liver injury with CCl₄, we referenced the previous protocol.³⁵ Briefly, at age of eight weeks, SPLucTg mice with a C57BL/6 background were intraperitoneally administered with 10% CCl₄ solution in corn oil (v/v) at a dose from 50 μ l to 300 μ l per 25 g mice on Day 0. Luciferase activity was monitored through *in vivo* imaging on day 1, 2, 3, 4, and 6.

In Vitro Luciferase Activity Assay and Western Blot Analysis

SPLucTg mice at eight to ten weeks of age were intraperitoneally injected with a 10% of CCl₄ solution in corn oils at a dose of 200 μ l/25g on Day 0. Mouse livers were isolated on day 1, 2, 3, 4, and 5 and homogenized using a PowerGen 125 homogenizer in 1× passive lysis buffer

(Promega; 0.5g liver tissues per ml buffer) for determining luciferase activity by luciferase assay or in Western blot lysis buffer (0.5g tissues per ml) for determining endogenous survivin expression by Western blot using survivin antibody (FL-142, Santa Cruz). The homogenized lysates were cleared by 18,000g centrifugation in 1.5 ml Eppendorf tube for 10 minutes at 4°C, and the resultant clear supernatant was used for luciferase assay or Western blots. Experimental procedures for data shown in the supplemental Table S1 at <http://ajp.amjpathol.org> are the same as above with the exception that a 5% of CCl₄ solution in corn oils at a dose of 450 μ l/25g was injected intraperitoneally on Day 0. Mice were euthanized and their livers were isolated 65 hours after CCl₄ injection. Detailed methods related to luciferase assays and Western blots were described in our previous publications.^{36,37}

Liver Tissue Collection and Immunohistochemistry Analysis

Liver tissues treated and untreated with CCl₄ (5% in coin oil, 400 μ l/25g) were isolated 65 hours after intraperitoneal injection. The isolated tissues were immediately preserved in three different formats: buffered formalin, zinc fixative (formalin free), and frozen in OCT frozen sectioning medium, followed by paraffin embedding. Preservation of liver tissues in three formats would provide great options for best immunohistochemistry (IHC) results with selected antibodies. Each of the paraffin-embedded liver tissues was multiply sectioned at 5 μ m and processed for IHC with primary antibodies for survivin (#2808, Cell Signaling), CD90 (#550543, BD Pharmingen), ABCG2 (ab24114, Abcam), CD133 (ab19898, Abcam). IHC analyses were performed following protocols described in our previous publications.^{38–40}

Statistical Analysis

The *in vivo* image data were analyzed using the LivingImage® 2.50.1 software in the package of the *in vivo* image system (Xenogen/Caliper LifeSciences, Hopkinton, MA). A two-group *t* test assuming equal variance was used for the data in the supplemental Figure S1 (at <http://ajp.amjpathol.org>). The significance (*P* value) was set at the nominal level of 0.05.

Results

Identification and Functional Validation of the SPLucTg Mice

A total of 20 offspring were born from 344 fertilized eggs microinjected with the human survivin promoter-driven luciferase reporter gene cassette. Genotype determination of the 20 transgenic mice using PCR with primers P1/P2 and P3/P4 (Figure 1A) identified five founder mice. Examples of these results are presented in Figure 1B. The founder mice were then crossed with wild-type

Figure 2. Human survivin promoter-driven luciferase activity (**green arrow**) during male SPlucTg mouse castration and regrowth with androgen replacement. **A:** Testosterone pellets were imbedded subcutaneously on day 0 (**black arrow**) onto SPlucTg mice, which were castrated 14 days ago. Luciferase activity was monitored by *in vivo* Imaging. The **red arrow** indicates testes showing luciferase activity before castration (day -15). **B:** The quantitative data derived from **A** are presented in a histogram format.

C57BL/6 mice to test germline transition of the transgene. Genotype determination using PCR indicated that four of the five founder lines successfully passed the transgene into offspring. Next, we determined whether the resultant F1 and F2 mice possessed a functional survivin promoter-luciferase reporter system. Our previous study identified testis as the only human organ that expresses high levels of survivin. We found that ~60% to 70% of spermatogonia cells showing high survivin expression.³⁸ Consistent with this observation, luciferase activity in SPlucTg mice was only detected in the testis (founder, F1 and F2) using an *in vivo* imaging system. Examples of three F1 SPlucTg mice are shown in Figure 1C (two males and one female).

The Human Survivin Promoter Can Be Activated in Castrated SPlucTg Mice after Androgen Treatment

Growing evidence indicates that normal cell proliferation is associated with a transient expression of survivin during various pathophysiologies.¹¹ It is known that mouse prostate tissues will shrink after castration for two weeks. However, exogenous supplement of androgen could stimulate tissue proliferation and regeneration. Therefore, we tested whether we could detect luciferase activity in castrated SPlucTg mice after androgen treatment. Our experiments showed that supplement of testosterone pellets to the castrated SPlucTg mice gives rise to a transient luciferase activity (Figure 2, A and B). This experiment suggests that SPlucTg mice possess an active and regulated reporter system.

Transient and Dose-Dependent Activation of the Survivin Gene Surrogate during Injury and Repair/Regeneration of the Liver in SPlucTg Mice

Although the expression of survivin in normal adult liver is usually undetectable, a previous report demonstrated that survivin expression is turned on during mouse liver injury and regeneration after CCl₄ administration.⁴¹ Therefore, we examined whether transient luciferase activity was apparent during CCl₄-induced SPlucTg mouse liver injury and regeneration. Our experiment showed that CCl₄-induced liver injury resulted in transient activation of the luciferase reporter system (Figure 3, A–H), further validating the physiological responsiveness of the survivin promoter-driven luciferase reporter gene cassette in SPlucTg mice. Importantly, luciferase activity intensity in response to CCl₄ in the SPlucTg mouse liver is dose-dependent (Figure 3).

Luciferase Activity Reflects the Expression of Endogenous Survivin in SPlucTg Mice

To determine the relationship between luciferase activity and endogenous survivin expression in the SPlucTg mice, we determined expression of both survivin and luciferase during CCl₄-induced liver injury and regeneration in SPlucTg mice over a period of five days after CCl₄ administration (Figure 4). Our results revealed that luciferase activity determined by *in vitro* luciferase activity assays, and endogenous survivin expression determined by Western blot analysis, mirror well. Similar to the expression pattern of endogenous survivin, *in*

Figure 3. Human survivin promoter-driven luciferase activity (green arrow) during CCl₄-induced SPLucTg mouse liver injury and repair processes. **A–D:** A 10% (v/v) of CCl₄ solution in corn oil was injected intraperitoneally on Day 0. Administered doses and the respective imaging day are indicated, respectively. The red arrow points the location of the testes, which are known to be positive for luciferase activity. **E–H:** The quantitative data derived from **A–D** are presented in histogram formats, respectively.

vivo imaging indicated that on Day 1 after administration of CCl₄, 90% mice show no bioluminescence ($n = 10$), whereas on Day 2, 80% of experimental mice show bioluminescence. Thereafter, the percentage of mice shows luciferase activity is in turn decreased from Day 3 to Day 5 (by Day 5 90% of the mice show no luciferase activity). Thus, our data revealed that there is a viable and sensitive luciferase reporter system in SPLucTg mice, which closely mimics endogenous survivin gene activity.

One critical issue for application of the SPLucTg mouse model is the variation of luciferase activity

among different individual mice. Here, we provide additional supplemental data derived from a larger cohort size to determine the mouse responsive rate (percentage) to treatment as well as possible variations of luciferase activity among individual mice resulted from the same treatment (see Supplemental Table S1 at <http://ajp.amjpathol.org>). The data in this table showed that 1) individual offspring produced from same homozygous SPLucTg mice show a minimal variation in luciferase activity on treatment; 2) luciferase activity variation in offspring from different homozygous SPLucTg mice in the same mouse line is also minimal; and

Figure 4. Association of luciferase activity with endogenous survivin expression in the CCl₄-induced liver injury and repair in SPLucTg mouse model (Line 2). A 10% of CCl₄ solution in corn oil at a dose of 200 μ l/25 g was injected intraperitoneally on Day 0. Mice were sacrificed and livers were isolated on days 1, 2, 3, 4, and 5, respectively. After the isolated livers were lysed, luciferase activity (**upper panel**, mean of triplicate assays from same sample) and survivin expression (**lower panel**) were determined using luciferase activity assays and Western blot analysis, respectively. Examples of mice from each day were shown. HeLa cell lysates was used as a survivin positive control, and the molecular size of the protein markers in the protein marker lane was indicated. m indicates male mice; f, female mice.

3) different mouse lines can show different sensitivity of responsiveness on CCl₄ treatment (see discussion).

Survivin Expression Overlaps with the Expression of Several Stem Cell Markers

Evidence indicates that survivin may play a role in stem cell (SC) function and may be a superior SC marker. For example, during colorectal⁹ and other types^{42,43} of carcinogenesis, survivin increases during the transition from adenoma with low dysplasia to high dysplasia/carcinoma, which involves abnormal β -catenin/TCF-4 signaling.^{44–46} To pursue the possibility that survivin expression overlaps with other SC markers and is a functional molecule during liver organ/tissue repair, we determined the expression of survivin, the liver-specific SC marker, CD90, and two other universal SC markers, CD133, ABCG2 in the CCl₄-induced liver repair mouse model. As shown in Figure 5, A and B, the expression of survivin, CD133, and CD90 colocalized around the vessel (active repairing area) in the injured but not uninjured liver. Moreover, individual survivin-positive cells show overlapping with CD133-, CD90-, and ABCG2-positive cells at the outside of active repairing areas, although we did not find cluster-positive cells around vessels for ABCG2. In contrast, there were no cells showing positive for these markers in the control liver. Furthermore, our experiments showed that only a small subset of cancer cells in cancer tissues express survivin (Figure 5C), which would be expected for a SC marker. Together, these data demonstrated that survivin could be a superior SC marker and may play a role in SC function.

Discussion

Issues Related to Generation of a Valid Transgenic Mouse Model

We have developed SPLucTg mice that express the luciferase reporter gene under the control of the human survivin promoter in a manner consistent with expression of endogenous survivin in various pathophysiological conditions. We used the transgene cassette DNA without the bacterial plasmid backbone DNA for generation of transgenic mice to preclude methylation effects, which are known to occur after random integration into the mouse genome.^{47,48} Such a methylation event could result in an epigenetic modification known as “methylation spread,” and might subsequently lead to methylation of the transgene cassette, resulting in silencing of transgene expression. Another fundamental issue is whether the human survivin promoter used in the study represents the behavior of the endogenous survivin gene promoter. To this point, we isolated a 4.5-kb DNA fragment as a putative entire human survivin promoter, which is evidenced by several observations. Our initial studies on the human survivin gene promoter regulation indicated that whereas the promoter at 2840 bp shows its promoter activity similar to its 1430 bp, its 6270-bp promoter neutralized its promoter activity by half,³¹ suggesting that there are additional inhibitory DNA motifs upstream of its 2840-bp promoter. Additionally, we found that there is similar promoter activity between its 6270-kb promoter and its 4500-bp promoter (unpublished observation), suggesting that the 4.5-kb survivin promoter likely represents the entire human survivin promoter. Importantly, our previous study indicated that the 38-bp DNA fragment just upstream of the survivin translation start site (ATG) is critical for cell cycle-dependent expression of survivin.³² The 4.5-kb survivin promoter used in this study includes this 38-bp DNA fragment as well. Therefore it contains all of the required DNA motifs for cell cycle-dependent regulation of survivin expression.^{32,33} Furthermore, this promoter was tested and verified in cancer cell-based *ex vivo* systems with a number of ligands including Hedyamin and Hoechst33342^{36,49} before being used for generation of transgenic mice. We chose to use the human survivin promoter instead of the mouse survivin promoter to generate a transgenic mouse model because we have much more information available on human survivin promoter regulation than on mouse survivin promoter regulation. Secondly, we hope that the human survivin promoter-driven luciferase reporter may reflect a closer situation by use of this model to mimic human tumor initiation, progression, and metastasis. However, we understand and agree that both survivin promoters (human versus mouse) have advantages and disadvantages for application of such a mouse model in various pathophysiological processes.

Additionally, we generated several founder-mouse lines to identify the most sensitive line for applications. Of the five lines, one failed to pass the transgene onto the next generation, and of the four remaining lines, Line 2 was found to have the most sensitive responses to

Figure 5. The pattern of survivin expression during liver injury and repair overlaps with stem cell markers. **A and B:** The treated condition is 5% CCl₄, 400 μ l/25g. Normal liver and CCl₄-treated liver tissues were isolated 65 hours after treatment and analyzed by IHC as shown. An example vessel is indicated by the **arrow**. The liver tissues in **A** were fixed with buffered formalin, which works well for survivin and CD133 antibodies. The liver tissues in **B** were fixed with zinc fixative, which works well for CD90 and ABCG2 antibodies. An image from both low magnification and high magnification in each situation is shown. **C:** Only a small subset of cancer cells express survivin. Human breast cancer (**upper panel**) and mouse carcinoma (**middle panel**) stained with survivin rabbit mAb (#2808, Cell Signaling). Mouse carcinoma stained with normal rabbit IgG.

treatment (refer to the supplemental Table S1 at <http://ajp.amjpathol.org> for more information) and is the best suitable line for mimicking endogenous survivin expression and regulation. The data in Supplemental Table S1 (at <http://ajp.amjpathol.org>) indicated that although different mouse lines show different sensitivity of responsiveness on CCl₄ treatment, individual mice from the same parental SPlucTg mice or from different parental SPlucTg mice in the same line show minimal and well acceptable variation in luciferase activity on treatment. Based on this information, experiments using offspring derived from the same line homozygous SPlucTg mice to cross with other tumor mouse models for studying the role and expression of survivin during tumorigenesis should have no potential problems related to batch-to-batch and individual-to-individual variations. Importantly, the luciferase activity variations shown in individual mice in Supplemental Table S1 (see <http://ajp.amjpathol.org>) likely reflect the endogenous survivin response to treatment at the defined dose and time point used. This notion was well supported by the data provided in Figure 4 in the article. Thus, the mouse model would provide a powerful tool for many

potential applications including those discussed in the article (see below).

Potential Pitfalls for Application of this Transgenic Mouse Model

Initial studies indicated that production of a high level of survivin mRNA is critical for maintenance of survivin protein expression during cell proliferation,³² that survivin expression is largely controlled by transcription,^{31,32} and that p53, as a major transcription factor, plays an important role in survivin transcription control in association with other transcription factors.^{50–53} Although increasing survivin protein expression may not always result from transcription, the presence of sufficient amounts of survivin mRNA is important. For example, although IGF-1/mTOR signaling does not directly stimulate the production of more survivin mRNA, it was shown that IGF-1/mTOR signaling needs survivin mRNA to increase translation.⁵⁴ Moreover, whereas the ubiquitin-proteasome pathway plays a role in rapid survivin degradation

in a cell cycle-dependent manner,⁵⁵ studies using a mouse survivin promoter-driven GFP-transgenic mouse model indicated that survivin gene expression is largely mitosis-independent *in vivo* but regulated by p53.³⁰ Based on these findings, we believe that the transgenic mouse model generated in this study will be uniquely useful for monitoring survivin gene activity during various pathophysiological processes. However, in certain physiological cases, this model could be inaccurate or even incorrect regarding survivin gene activity. For example, it was reported that cell proliferation and maturation in the bone marrow is accompanied by survivin expression.^{56–59} We failed to detect the presence of luciferase activity in the bone marrow using *in vivo* and *ex vivo* imaging or *in vitro* luciferase assay. However, we are able to easily detect survivin mRNA and protein expression in the mouse bone marrow by real-time RT-PCR and western blots, which is consistent with our previous finding using immunohistochemistry.³⁸ As an additional note, it appears that there is a trend that luminescence intensity increases in testes with increasing CCl₄ dose as shown in Figure 3. This phenomenon suggests that CCl₄ administration may injure testis and stimulate testis cells to express survivin as well. All of these interesting observations are currently under investigation in our laboratory.

Previously, our lab found that estrogen induces survivin expression in breast cancer cells,⁶⁰ and both breast and ovarian cancer tissues express survivin. However, there is no survivin expression in human normal breast and ovary tissues.³⁸ To delineate the mechanism for survivin negativity in human breast and ovary tissues, we used the p53 negative and ER α low Saos-2 cells to determine whether wild-type and mutant p53 can show different regulation of survivin expression with and without estrogen treatment. We found that wild-type p53 but not mutant p53 can effectively block ER α -mediated up-regulation of survivin promoter activity in the present or absence of estrogen (see the supplemental Fig. S1 at <http://ajp.amjpathol.org>). These data imply a possible mechanism for survivin negativity in human breast and ovary tissues through suppression by wild-type p53.

Issues Related to Versatile Applications of the Generated Mouse Model as a Tool

Although evidence indicates a role for survivin in the promotion of tumor invasion, metastasis, and tumor recurrence,^{61–65} the precise role for survivin in these processes has not been well defined *in vivo*. This is in part because the expression of survivin cannot be monitored in living animals without sacrificing mice during these processes. The SPLucTg mouse model generated in this study will help to fill this gap and can be used to monitor the expression of survivin during cancer initiation, tumor progression, and metastasis in living animals by *in vivo* imaging. For example, one could use a carcinogen such as DMBA to initiate tumor and then apply a tumor promoter such as PMA to further stimulate tumor development using the generated mouse model for the studies. Alternatively, one could cross the mouse model gener-

ated in this study with other cancer-susceptible mouse models (such as the TRAMP mouse model or the p53/Rb double knockout mouse model) to create hybrid cancer mouse models to perform the studies. In short, the use of this animal model should facilitate elucidation of the expression and possible role for survivin during cancer initiation, tumor progression, and metastasis.

An additional issue relevant to the application of this animal model is that during liver injury and repair processes, our IHC experiments demonstrated that the expression of survivin aligns closely with the stem cell markers (CD133, CD90 [a liver-specific stem cell marker], and ABCG2), whereas ABCG2 does not overlap with CD133 and CD90 (Figure 5, A and B). The observation raises an important notion as to whether survivin might represent a superior stem cell marker and may play an important role in stem cell biology. Consistent with this possibility, our data (Figure 5C) in this report as well as our previous studies indicated that survivin is expressed in a very small subset of cell populations in normal proliferative tissues³⁸ and in cancer tissues, whereas other studies also suggest a possible involvement of survivin in stem cells.^{44,66,67} Furthermore, computer analysis of the death-from-cancer signature genes indicating that cancer cells with a stem cell-like expression profile possess three characteristics: increased IAP expression, activated mitotic spindle check point proteins, and elevated expression of cell cycle control proteins.⁶⁸ Importantly, survivin is a key member of the IAP family and possesses all these characteristics—apoptosis inhibition, mitosis control, and cell cycle promotion.^{1–3} Together, survivin appears to be a stem cell marker, and tracking luciferase activity by *in vivo* imaging may be able to track stem cell activity. We propose that in terms of using survivin as a stem cell marker, our animal model may be useful in stem cell research especially in consideration of recent observations from our studies and others that tumor cell lines may not be the best model system to study cancer stem cells.⁶⁹ That is, tumor-initiating cancer cell lines possess a large subset of cell population that can initiate tumors *in vivo* allo/xenografting models.⁶⁹ Intriguingly, it was proposed that stem cells may not be a particular cell type but an emergent property of cell lineages under feedback control.^{70,71} Therefore, monitoring survivin gene activity may be a better strategy to trace cancer stem cells.

In sum, the model system generated in this study may have versatile applications in various pathophysiological processes including cancer and stem cell research.

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