Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 342 (2006) 300–309
BBRC
www.elsevier.com/locate/ybbrc

# Overexpression of ABIN-2, a negative regulator of NF-κB, delays liver regeneration in the ABIN-2 transgenic mice

Chao-Chin Li [a], Chen-Kung Chou [b,c,d], Ming-Hseng Wang [e], Ting-Fen Tsai [a,c,d,*]

[a] *Faculty of Life Sciences and Institute of Genetics, National Yang-Ming University, Taipei, Taiwan*
[b] *Department of Life Science, Chang Gung University, Taoyuan, Taiwan*
[c] *Department of Medical Research and Education, Taipei Veterans General Hospital, Taiwan*
[d] *Division of Molecular and Genomic Medicine, National Health Research Institute, Zhunan, Taiwan*
[e] *Laboratory Animal Center, Tzu-Chi University, Hualien, Taiwan*

Received 6 December 2005
Available online 2 February 2006

**Abstract**

Activation of NF-κB is one of the earliest responses at the start of liver regeneration, and is required for hepatocyte cell cycle progression. The A20-binding inhibitor of NF-κB activation-2, ABIN-2, is an inhibitor of NF-κB. However, its effects on hepatocyte cell cycle progression are not known and its involvement in liver regeneration has not been explored. In this study, the temporal expression pattern of the mouse ABIN-2 was studied during liver regeneration induced by partial hepatectomy. We demonstrate that ABIN-2 is rapidly and transiently induced, and expression peaked at around 8 h post-hepatectomy. To test that the inducible expression of ABIN-2 serves to regulate NF-κB during liver regeneration, transgenic mice overexpressing human ABIN-2 protein in the liver were generated. Our transgenic data demonstrated that overexpression of ABIN-2 inhibited NF-κB nuclear translocation, which peaked at around 2–4 h post-hepatectomy, and this led to an impairment of the G1/S transition as well as a delay in hepatocyte cell cycle progression of the regenerating liver. In addition, overexpression of ABIN-2 specifically inhibited endogenous ABIN-2 mRNA induction, suggesting a negative feedback mechanism for ABIN-2 expression. In conclusion, ABIN-2 may function as a negative regulator that downregulates NF-κB activation during liver regeneration.


*Keywords:* ABIN-2; NF-κB; Liver regeneration; Transgenic mice; Partial hepatectomy

Activation of nuclear factor-κB (NF-κB) plays a pivotal role in many biological processes [1]. NF-κB has been implicated in liver development and regeneration. NF-κB consists of two polypeptides, p65/RelA and p50. Mice deficient in p65/RelA exhibit severe phenotypic effects. Disruption of the p65/RelA subunit of NF-κB leads to embryonic lethality during gestation, concomitant with a massive degeneration of the liver by apoptosis [2]. Inhibition of NF-κB in hepatocyte cell lines blocks TNF-induced proliferation and sensitizes these cells to apoptosis [3,4]. Furthermore, activation of NF-κB is one of the earliest responses that has been detected at the start of liver regeneration after liver injury or partial hepatectomy (PH), suggesting that NF-κB may play a role in making hepatocytes competent to proliferate during liver regeneration [5,6]. NF-κB is localized primarily in the hepatocytes of the regenerating liver as demonstrated by mRNA induction of IκBα, which is one of the target genes of NF-κB, in hepatocytes in situ [6].

In resting cells, NF-κB exists in the cytoplasm in an inactive form associated with one of IκB inhibitory proteins, IκBα or IκBβ [7]. After stimulation of cells, activation of NF-κB is achieved through proteolytic degradation of IκB in the cytoplasm. The active NF-κB is then free to be translocated to the nucleus, where it regulates the transcription of the NF-κB-responsive genes by interacting with κB binding sites [7]. In addition to being

* Corresponding author. Fax: +886 2 2828 0872.
*E-mail address:* tftsai@ym.edu.tw (T.-F. Tsai).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.01.114

negatively regulated by IκB, NF-κB-dependent gene expression is also inhibited by the zinc finger protein A20 in a variety of cell types in response to various stimuli [8,9]. A20 is a potent inhibitor of NF-κB signaling [10]. The crucial physiological functions of A20 were confirmed by studies in A20-deficient mice. Pathological examination indicated hepatocyte loss in A20-deficient livers. This phenotype correlates with enhanced sensitivity to TNF-mediated apoptosis and prolonged NF-κB activation of A20-deficient cells [11]. The A20-binding proteins may contribute to the underlying mechanism of NF-κB inhibition by A20. The A20-binding inhibitor of NF-κB activation-2, ABIN-2, was originally identified in a yeast two-hybrid screening using A20 as the bait [12]. Ectopic expression of ABIN-2 has been shown to considerably inhibit NF-κB activation in response to TNFα or IL-1, suggesting that ABIN-2 may be a downstream effector of A20 that mediates inhibitory function of A20 [13,14].

Previous evidence has indicated that the activation of NF-κB during liver regeneration after PH appears to be a required event to allow normal cell cycle progression [15,16]. Although ABIN-2 is an inhibitor of NF-κB, its effects on hepatocyte cell cycle progression are not known and its involvement in liver regeneration has not been explored. In this study, we demonstrate the rapid and transient induction of ABIN-2 post-hepatectomy. It is likely that the inducible expression of ABIN-2 serves to regulate NF-κB during liver regeneration. To test this possibility, we have generated transgenic mice overexpressing human ABIN-2 protein in the liver and studied the phenotypic effects on liver regeneration.

## Materials and methods

*Partial hepatectomy and liver regeneration.* Partial hepatectomy (PH) was performed in wild-type and transgenic male mice at 9–10 weeks of age. About 70% of the liver including the median and left lateral lobes was removed after anesthetized with 50 mg/kg ketamine intraperitoneally [17]. Regenerated liver tissue was harvested at different time intervals. Serum alanine aminotransferase (ALT) was analyzed using FUJIFILM DRI-CHEM 3500s. There are three to five survived mice for each time point in each group of mice.

*The ABIN-2 transgenic construct.* The PEPCKex vector contains the human PEPCK promoter, a 0.3-kb ApoAI intron and poly(A) signal of human growth hormone gene provided by Dr. Brendan Lee (Baylor College of Medicine, Houston, TX). The PEPCK-ABIN2 transgenic construct was generated by inserting the human ABIN2 cDNA (NCBI Accession No. NM_024309) into PEPCKex.

*Generation of transgenic mice.* The ABIN-2 transgenic mice were generated by pronucleus microinjection of FVB/N fertilized eggs [18]. The transgenic mice were bred in a specific pathogen-free facility. Genomic DNA from tails was isolated [19]. The genotypes of mice were determined by PCR and Southern analysis [20]. To detect the ABIN-2 transgene, the 563-bp fragment was amplified by PCR with cycling conditions of 94 °C for 1 min, 62 °C for 30 s, and 72 °C for 40 s for 30 cycles using primers 5′-GGAGAGAAGTCCTGACCAGTCG-3′ and 5′-CCATAAGCTCTA ATGCGTCGGC-3′.

*RNA analysis.* Total RNA was isolated using TRIzol Reagent (Life Technology). Northern blot and slot blot hybridization were performed [19]. The cDNA probes for mouse ABIN-2 (NCBI Accession No. NM_139064, from base 323 to 1105), A20 (NCBI Accession No. NM_009397, from base 840 to 1660), cyclin D1 (NCBI Accession No. NM_007631, from base 189 to 903), and Pcna (NCBI Accession No. NM_011045, from base 174 to 856) were generated by RT-PCR. The cDNA probes for IκBα (RIKEN clone H3026A08, NCBI Accession No. BG064981), cdk2 (RIKEN clone H3117F10, NCBI Accession No. BG073019), and cdk1 (RIKEN clone H3024E04, NCBI Accession No. BG064846) were obtained from the NIA mouse 15K cDNA library. For semi-quantitative RT-PCR of mouse endogenous ABIN-2 mRNA, dilutions of cDNA were amplified for 12 cycles at 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min to achieve optimal condition for linearity of the signal strength. The 703-bp RT-PCR products were analyzed by Southern hybridization. The RT-PCR primers for mouse ABIN-2 were 5′-CTAAAGAGGCGGCAGGTCCCTC-3′ and 5′-CAAGATGACCTT CCAGTGAC-3′.

*Western blot analysis and immunohistochemical detection.* Western blot and immunohistochemical (IHC) staining of paraffin-embedded liver sections (3 μm) were performed [21]. The subcellular location of the NF-κB and hepatocyte expression of the ABIN-2 protein were examined using an antibody against the p65/RelA subunit of NF-κB (Santa Cruz, sc-273-G) and a polyclonal antibody for human ABIN-2, respectively, and detected using a LSAB kit (DakoCytomation).

*DNA synthesis and mitotic index.* S-phase cells were measured by bromodeoxyuridine (BrdU) incorporation into nuclei. Mice were injected with BrdU (Sigma, 100 mg/kg body weight) intraperitoneally 2 h before sacrifice. The IHC analysis was performed on paraffin-embedded liver sections using anti-BrdU antibodies (DAKO). For the mitotic index, the number of mitotic hepatocytes with mitotic figures of visible condensed chromosomes were counted.

*Statistics.* Results are presented as means ± SD. Student's $t$ test was used to calculate probability values. When analyzing statistical differences between ABIN-2 transgenic and wild-type mice, a value of $p < 0.05$ was considered significant.

## Results

### *Rapid and transient induction of ABIN-2 and A20 in the regenerating liver of wild-type mice post-hepatectomy*

To identify the potential regulatory roles of ABIN-2 with NF-κB in liver regeneration, temporal expression pattern of the mouse ABIN-2 mRNA was determined in wild-type mice after PH. A previous report has shown that ABIN-2 mRNA was expressed at low level in the adult mouse liver [12]. However, our data demonstrated that ABIN-2 mRNA was rapidly induced by 2 h, and high levels were maintained between 6 h and 12h post-hepatectomy (Fig. 1A). Peak induction of ABIN-2 mRNA was at 8 h post-hepatectomy and this was about 6.5-fold higher than the basal level of resting liver (Fig. 1B). Expression of ABIN-2 mRNA rapidly decreased to the basal level by 24 h post-hepatectomy (Figs. 1A and B). A previous report has shown that A20 was induced following stimulation with TNFα in primary human hepatocytes and the HepG2 cells [22]. IκBα is also rapidly induced in the regenerating liver [23]. Since A20 and IκBα are both negative regulators of NF-κB, the temporal expression patterns of the mouse A20 and IκBα were examined and compared to ABIN-2. The A20 and IκBα were rapidly induced with peak expression at 4 and 8 h, respectively, post-hepatectomy. Like ABIN-2, expression of the A20 and IκBα mRNA rapidly decreased to a basal level by 24 h post-hepatectomy (Figs. 1A and B). The phenomenon of rapid and transient induction of mouse ABIN-2

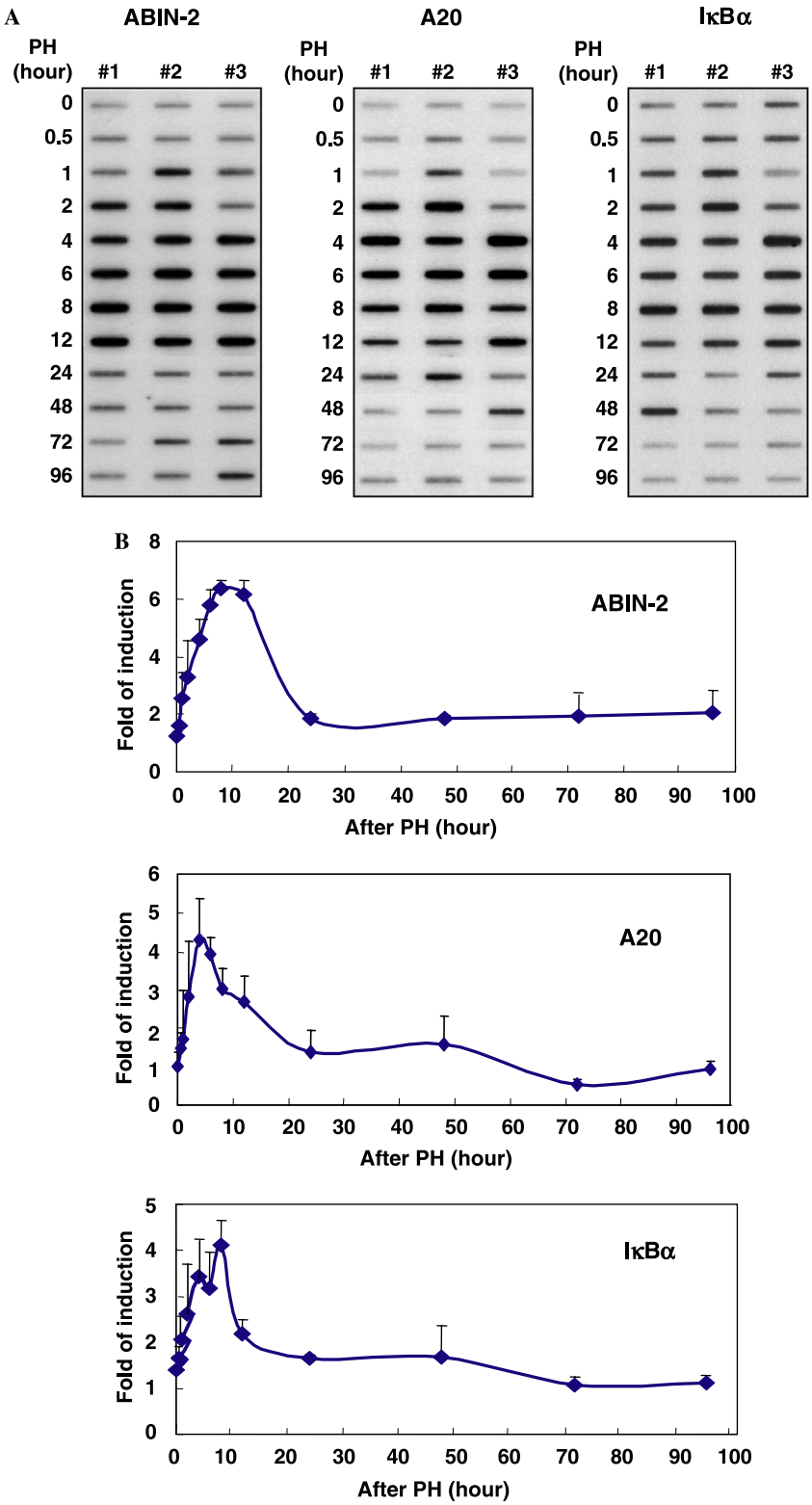


Fig. 1. ABIN-2 mRNA was rapidly and transiently induced in the regenerating liver of wild-type mice. (A) Slot blot hybridization of mRNA expression for mouse ABIN-2, A20, and IκBα gene post-hepatectomy. PH, partial hepatectomy. Five micrograms of total RNA from each sample was used for slot blot hybridization using mouse cDNA as the probes. Three individual male mice (#1, #2, and #3) were sacrificed at the indicated time intervals post-hepatectomy. All RNA samples were quantified using a spectrophotometer and further confirmed by gel electrophoresis in a standard parallel experiment for all slot blots. (B) Quantification of the slot blot hybridization signals of ABIN-2, A20, and IκBα mRNA induction in the regenerating livers. 28S rRNA was used as a control for all RNA hybridization. Quantification of the hybridization signal was analyzed using ImageQuant software from Molecular Dynamics. The results are presented as the fold of induction relative to PH 0 h and are shown as means ± SD.

post-hepatectomy suggested that ABIN-2 might have a regulatory role linked to NF-κB in liver regeneration.

*Generation of the ABIN-2 transgenic mice*

We generated transgenic mice overexpressing ABIN-2 in the liver to test whether ABIN-2 plays a role in the regulation of NF-κB during liver regeneration. The plasmid used to generate the ABIN-2 transgenic mice is shown in Fig. 2A. Four founders of the PEPCK-ABIN2 transgenic mice were generated. The transgenic copy number of line A0061, A0063, A0059, and A0089 is about 20, 10, 3, and 2, respectively, by Southern analysis (Fig. 2B). Expression of the human ABIN-2 mRNA was detected mainly in the

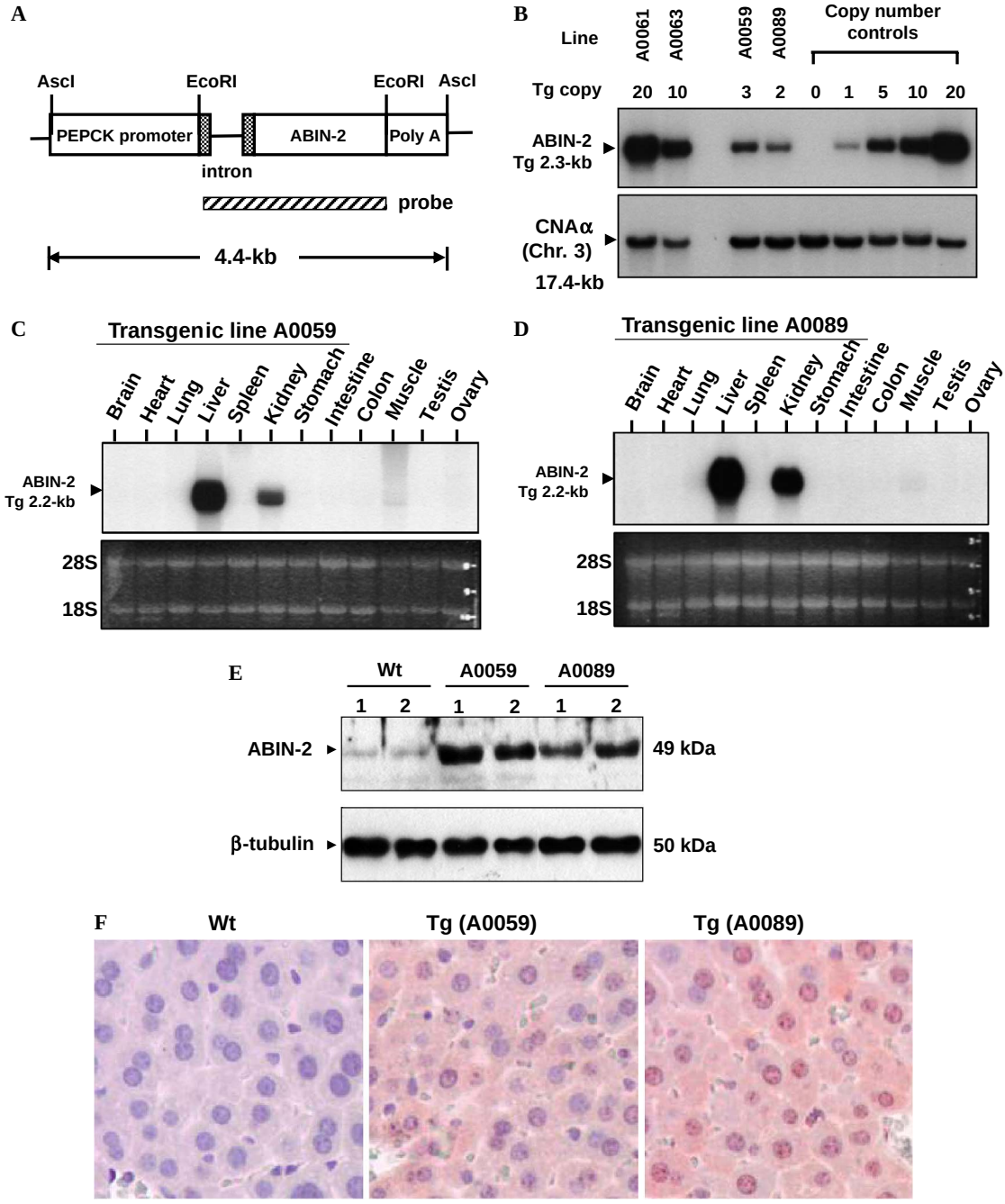


Fig. 2. Generation of the ABIN-2 transgenic mice. (A) The PEPCK-ABIN2 transgenic construct. The human ABIN-2 cDNA was driven by the PEPCK promoter. The *Asc*I sites were used to excise the 4.4-kb insert for pronucleus microinjection. (B) Southern analysis of transgene (Tg) copy number for lines A0061, A0063, A0059, and A0089. Genomic DNA was digested with *Eco*RI for Southern blot hybridization. The probe for ABIN-2 transgene was a 2.3-kb *Eco*RI fragment containing the intron and human ABIN-2 cDNA. The hybridization signal of calcineurin Aα (CNAα) located on mouse chromosome 3 was used as internal control for genomic DNA loading. (C,D) Northern blot analysis of the human ABIN-2 mRNA expression in transgenic lines A0059 and A0089. (E) Western blot detection of ABIN-2 protein using a polyclonal antibody against human ABIN-2. (F) IHC detection of the ABIN-2 protein in the livers of wild-type (Wt), transgenic lines A0059 and A0089. All of the liver samples were obtained from 2-month-old male mice.

kidney of lines A0061 and A0063 and these lines were not further investigated in this study. Strong expression of the human ABIN-2 mRNA was detected in the livers of lines A0059 and A0089, with a lower level detected in the kidney (Figs. 2C and D). Expression of the ABIN2 protein was demonstrated by Western blot and IHC staining. The human ABIN-2 antibody also recognizes the endogenous mouse ABIN-2 protein as weak signal and this was also detected in the wild-type liver samples (Fig. 2E). However, significantly elevated levels of the protein were detected in lines A0059 and A0089, indicating that the human ABIN-2 protein was indeed overexpressed in the transgenic liver (Fig. 2E). Furthermore, IHC staining confirmed that the ABIN-2 protein was detected in the hepatocytes of transgenic livers of both transgenic lines (Fig. 2F).

*Overexpression of ABIN-2 inhibits NF-κB nuclear translocation in the hepatocytes of regenerating liver*

To determine the effect of ABIN-2 overexpression on NF-κB activation post-hepatectomy, we analyzed the subcellular distribution of NF-κB in hepatocytes and non-parenchymal cells. IHC staining revealed that the p65/RelA subunit of NF-κB was localized in the cytoplasm of the hepatocytes in both wild-type and ABIN-2 transgenic mice before PH. A peak in the rapid nuclear accumulation of the p65/RelA in hepatocytes was detected between 2 and 4 h post-hepatectomy in the wild-type mice (Fig. 3). Previous reports have shown that the peak of NF-κB nuclear translocation was detected at 0.5–1 h post-hepatectomy in rat [5,6]. This temporal difference may possibly be attributable to the different animal model investigated. Our data indicated that in both of the ABIN-2 transgenic lines, nuclear translocation of p65/RelA in the hepatocytes was markedly decreased at 2 and 4 h post-hepatectomy (Fig. 3). Nuclear staining of the p65/RelA could be classified into three levels: high (++), intermediate (+), and negative (−). There were 66.7% and 33.3% of mice with hepatocyte nuclear staining of p65/RelA classified into high (++) and intermediate (+), respectively, in wild-type mice at 2 and 4 h post-hepatectomy (Table 1). In contrast, IHC staining revealed that hepatocyte nuclear staining of p65/RelA for majority of the ABIN-2 transgenic mice was classified into the intermediate (+) or negative (−)

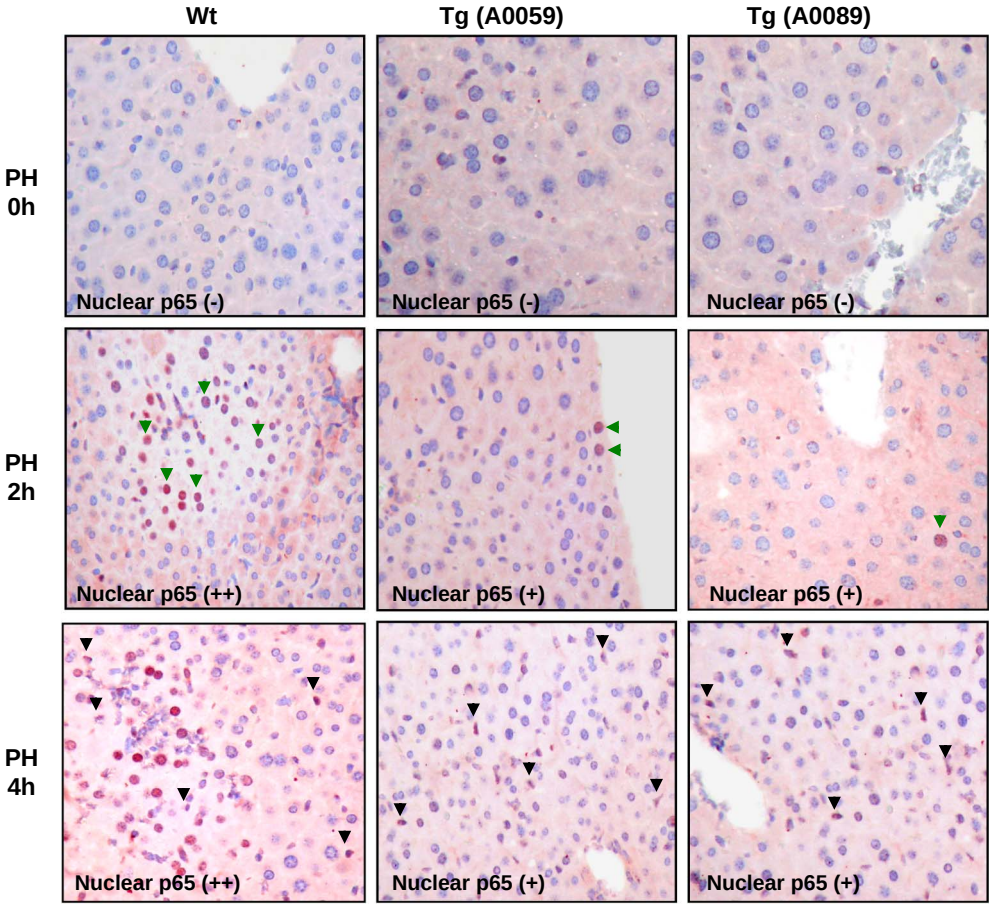


Fig. 3. Inhibition of nuclear translocation of the NF-κB in the ABIN-2 transgenic livers post-hepatectomy. Nuclear translocation of the p65/RelA subunit of NF-κB was examined by IHC staining using p65/RelA specific antibody. Nuclear staining of p65/RelA was classified into three levels: high (++), intermedium (+), and negative (−). Representative photomicrographs are shown. Magnification, 100×. Green arrows indicate hepatocytes with positive nuclear staining of p65/RelA and black arrows indicate non-parenchymal cells with positive nuclear staining of p65/RelA.

Table 1
Comparison of nuclear translocation of the p65/RelA subunit of NF-κB post-hepatectomy

| PH (h) | Nuclear p65/RelA | Wild type | Tg (A0059) | Tg (A0089) |
|---|---|---|---|---|
| 0 | ++ | 0% (0/3) | 0% (0/3) | 0% (0/3) |
| | + | 0% (0/3) | 0% (0/3) | 0% (0/3) |
| | − | 100% (3/3) | 100% (3/3) | 100% (3/3) |
| 2 | ++ | 66.7% (4/6) | 0% (0/6) | 0% (0/6) |
| | + | 33.3% (2/6) | 83.3% (5/6) | 66.7% (4/6) |
| | − | 0% (0/6) | 16.7% (1/6) | 33.3% (2/6) |
| 4 | ++ | 66.7% (2/3) | 0% (0/3) | 0% (0/4) |
| | + | 33.3% (1/3) | 33.3% (1/3) | 75% (3/4) |
| | − | 0% (0/3) | 66.7% (2/3) | 25% (1/4) |

Tg, ABIN-2 transgenic mice. Nuclear staining of p65/RelA could be classified into three levels: strong (++), intermedium (+), and negative (−).

classes for both transgenic lines at 2 and 4 h post-hepatectomy (Table 1). NF-κB nuclear translocation of non-parenchymal cells was evident 4 h post-hepatectomy. No significant differences were observed in NF-κB nuclear translocation in non-parenchymal cells of the wild-type and ABIN-2 transgenic mice (Fig. 3). This is not unexpected since the transgenic ABIN-2 protein is overexpressed mainly in the hepatocytes. The transgenic data clearly demonstrated that overexpression of the ABIN-2 inhibits NF-κB nuclear translocation of hepatocytes in the regenerating liver.

### *A delay in hepatocyte cell cycle progression and impairment of the G1/S transition, but no apoptosis in the regenerating livers of the ABIN-2 transgenic mice*

To study whether inhibition of the NF-κB nuclear translocation leads to delay or impairment of hepatocyte cell cycle progression post-hepatectomy, DNA synthesis and mitosis in hepatocytes were analyzed in detail for ABIN-2 transgenic line A0089. DNA synthesis was assessed by BrdU incorporation as detected by IHC staining (Figs. 4A and B). For the wild-type mice, the majority of the BrdU-positive hepatocytes were detected at 1.5- to 2-day post-hepatectomy (Fig. 4C). However, there was a significantly reduced percentage of the BrdU-positive hepatocytes and a delay of DNA synthesis detectable in the ABIN-2 transgenic mice (Fig. 4C). In wild-type mice, the number of mitotic figures peaked at day 1.75 and decreased thereafter until day 4 post-hepatectomy (Fig. 4D). A decrease in the mitotic figures and a delay of mitosis were also observed in the ABIN-2 transgenic mice (Fig. 4D). Minor histological changes were detected in the regenerating livers of the ABIN-2 transgenic mice. In wild-type mice, cytoplasmic vacuolation of hepatocytes transiently occurs around day 1 and usually had recovered by day 1.5 post-hepatectomy (data not shown). However, vacuolation and fat accumulation were still overt at day 1.75 in the ABIN-2 transgenic livers post-hepatectomy (Fig. 4B). This histological change might be due to a delay in the hepatocyte cell cycle progression. To assess liver cell damage after PH, serum ALT levels were measured. The ALT levels were significantly increased at day 1 and returned to normal levels at around day 4 post-hepatectomy in both wild-type and ABIN-2 transgenic mice (Fig. 4E). The serum ALT levels for both types of mice were similar, indicating no severe liver damage had occurred in the ABIN-2 transgenic mice post-hepatectomy compared with wild-type mice. At day 1 post-hepatectomy, occasional cell death was observed in both wild-type and ABIN-2 transgenic mice. However, no apoptotic or necrotic cells were detected in the ABIN-2 regenerating livers other than on day 1; and this was confirmed using TUNEL assays (data not shown). Finally, the overall ability of the liver to regenerate was determined by measuring the ratio of liver to body weight. The ratios of regenerated liver to body weight for the ABIN-2 transgenic mice were significantly lower than those of the wild-type mice 7 days post-hepatectomy (Fig. 4F), indicating that liver regeneration was delayed in the ABIN-2 transgenic mice.

To study whether the observed decrease in hepatocyte DNA synthesis and mitosis in the ABIN-2 transgenic livers might reflect an impaired progression of hepatocytes across the G1/S transition, we measured the steady-state mRNA levels of various cell cycle associated markers. There was no obvious difference in the induction of cyclin D1 (G1 phase) expression in the wild-type and ABIN-2 transgenic mice (Fig. 5A). However, induction of cdk2 (G1/S phase), Pcna (S/G2/M phase), and cdk1 (G2/M phase) was remarkably decreased in the ABIN-2 transgenic mice compared to those of the wild-type mice (Figs. 5B–D). Taken together, our data indicated that overexpression of ABIN-2 led to impairment of the G1/S transition and a delay in hepatocyte cell cycle progression for the regenerating liver post-hepatectomy.

### *Overexpression of ABIN-2 inhibited endogenous ABIN-2 mRNA induction post-hepatectomy*

To examine whether overexpression of the ABIN-2 transgene affects endogenous ABIN-2 induction, we performed semi-quantitative RT-PCR using primers specific for the mouse ABIN-2 mRNA. Quantitative data indicated that induction of the endogenous ABIN-2 mRNA was suppressed in the ABIN-2 transgenic livers compared to wild-type mice post-hepatectomy (Fig. 6A). However, induction of the A20 and IκBα mRNA was not affected by ABIN-2 overexpression when studied using quantitative slot blot hybridization (Figs. 6B and C). This suggests that there might be negative feedback regulation controlling the transient induction of ABIN-2 expression during liver regeneration.

## Discussion

### *ABIN-2 is rapidly and transiently induced, and may function as a negative regulator of NF-κB during liver regeneration*

Stringent control of the rapid induction and then turn-off of NF-κB activation is required for regulation of the

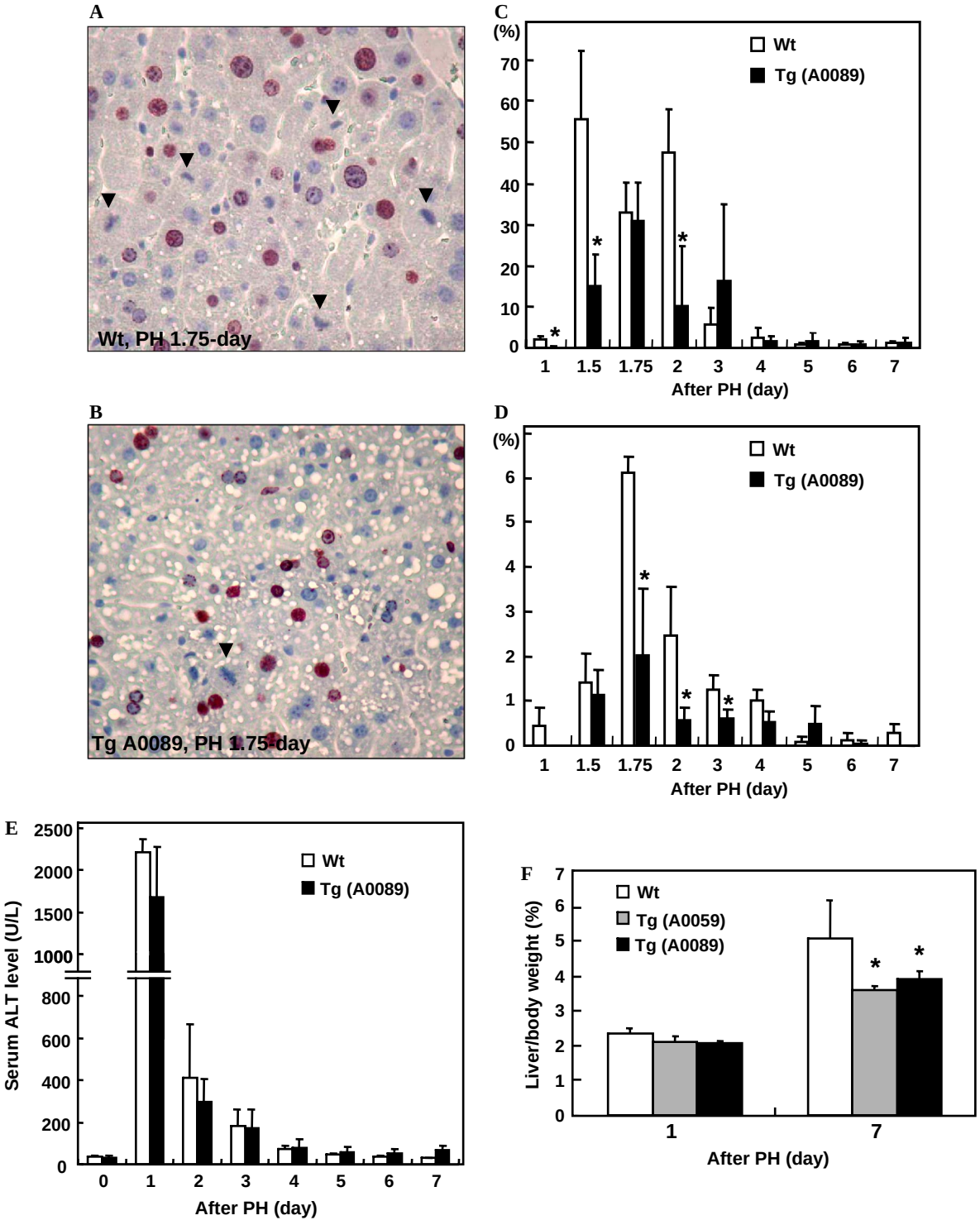


Fig. 4. Decreased/delayed DNA synthesis and mitosis in the regenerating livers of the ABIN-2 transgenic mice. (A,B) Representative photomicrograph of the BrdU IHC staining prepared from the regenerating liver of wild-type (Wt) and ABIN-2 transgenic mice (A0089) at 1.75-day post-hepatectomy. The BrdU-positive nuclei were stained a brown color. Arrows indicate mitotic hepatocytes undergoing chromosome segregation. (C) DNA replication as monitored by BrdU incorporation. BrdU-positive hepatocytes were quantitated by counting about 1000 hepatocytes for each experimental group. (D) Mitotic index of the regenerating liver. About 1000 hepatocytes for each experimental group were examined for the present of mitotic figures. The mean for each time point was expressed as a percentage of total hepatocytes counted. (E) Serum ALT levels. (F) Liver/body weight ratio. Liver and whole body weights were determined for the ABIN-2 transgenic mice and wild-type mice sacrificed at 7 days post-hepatectomy. The results are shown as means ± SD. When analyzing statistical differences between ABIN-2 transgenic and wild-type mice, $*p < 0.05$ was considered significant.

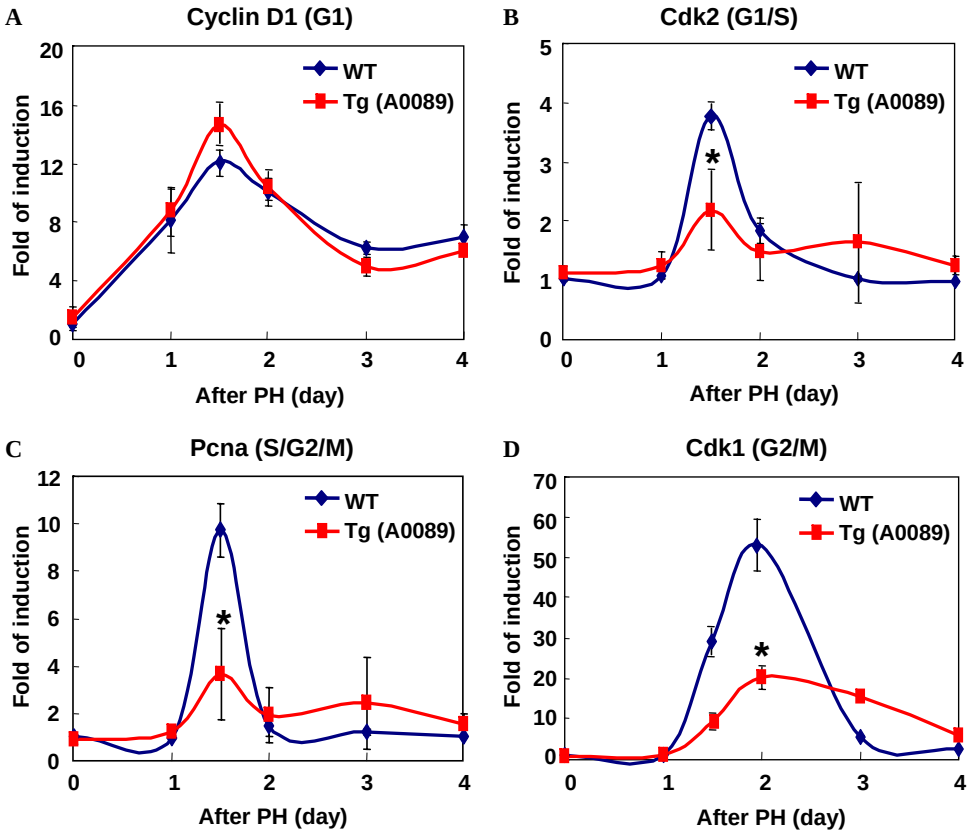


Fig. 5. Expression of the cell cycle associated gene cyclin D1 (A), cdk2 (B), Pcna (C), and cdk1 (D) mRNA in the wild-type and ABIN-2 transgenic mice post-hepatectomy. The methods for the quantification of the mRNA expression by slot blot hybridization are the same as Fig. 1. The results are presented as fold of induction relative to PH 0 h and are shown as means ± SD. When analyzing statistical differences between ABIN-2 transgenic and wild-type mice, $*p < 0.05$ was considered significant.

biological processes involved in liver development and regeneration. Lack of NF-κB activation leads to severe phenotypes involving embryonic lethality and liver degeneration has been demonstrated in mice deficient in either IKKβ [24,25] or IKKγ [26]. On the other hand, failure to downregulate NF-κB transcriptional activity results in chronic inflammation of liver and hepatocyte loss as demonstrated in A20-deficient mice [11]. In this study, we showed that A20 and ABIN-2, like the negative regulator IκBα, are rapidly and transiently induced in the regenerating liver post-hepatectomy. IκBα and A20 are both negative regulators involved in a negative feedback loop for NF-κB activation and are transcriptionally controlled by NF-κB through κB sites. The κB sites in the IκBα and A20 promoter are located within 100 base pairs upstream of the transcriptional start site [27,28]. However, no consensus κB site can be found in the ABIN-2 promoter region after searching about 2 kb of the upstream DNA sequence, suggesting that ABIN-2 may be not a direct target gene of NF-κB.

### *ABIN-2 overexpression inhibits NF-κB nuclear translocation and delays hepatocyte cell cycle progression post-hepatectomy*

It has been demonstrated that A20 targets the receptor-interacting protein (RIP) for proteasomal degradation to terminate NF-κB activation signaling [29]. An A20-interacting protein, such as ABIN-2, may have a role in the regulation of the inhibitory effects of A20 on NF-κB activation. A20 mutants with a complete loss of the ABIN-2 binding property correlate with a complete loss of A20's ability to inhibit TNF-induced NF-κB activation [30]. Previously, Liu et al. showed that ABIN-2 specifically forms a stable complex with IKKγ. ABIN-2 and RIP share a common structural feature that is essential for IKKγ binding, suggesting that ABIN-2 exerts its inhibitory function by competing specifically for the binding site in IKKγ with RIP, thus blocking RIP-induced NF-κB activation [14]. Consistent with previous observation that ABIN-2 functions at a level that is upstream of the IKK complex, we demonstrated that overexpression of the ABIN-2 inhibited NF-κB nuclear translocation and this is concomitant with the delayed cell cycle progression of hepatocytes post-hepatectomy. A previous study by Chaisson et al. showed that hepatocyte-specific inhibition of NF-κB through the overexpression of an inducible IκBα mutant did not lead to apoptosis or decreased hepatocyte proliferation post-hepatectomy [31]. The histological aspect of our data is similar to the findings of Chaisson et al. and shows that the inhibition of NF-κB nuclear translocation does not lead to apoptosis or cell death post-hepatectomy. However, a phenotypic effect on the cell cycle progression of

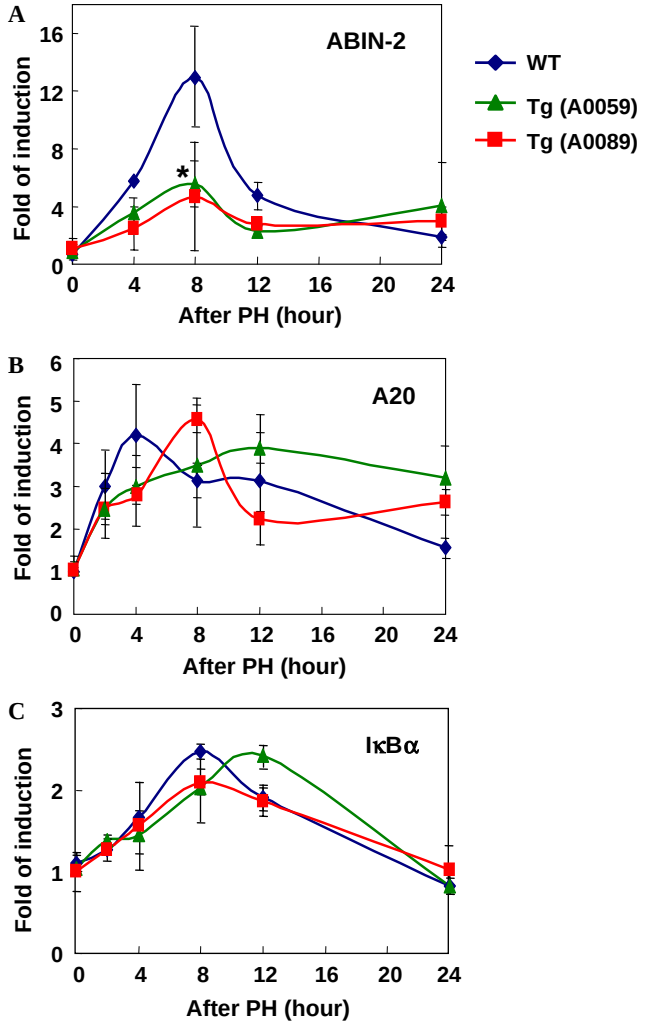


Fig. 6. Expression of the endogenous ABIN-2 (A), A20 (B), and IκBα (C) mRNA in the wild-type and ABIN-2 transgenic mice post-hepatectomy. The methods for the quantification of the mRNA expression by slot blot hybridization are the same as Fig. 1. The results are presented as fold of induction relative to PH 0 h and are shown as means ± SD. When analyzing statistical differences between ABIN-2 transgenic and wild-type mice, $*p < 0.05$ was considered significant.

hepatocyte was detected in the ABIN-2 transgenic mice. Apparently, there is a selective effect of ABIN-2 on cell proliferation, but not apoptosis, through inhibition of NF-κB nuclear translocation. Accordingly, the ABIN-2 transgenic mouse model offers the opportunity to study the molecular mechanism involved in the brief activation of NF-κB after PH, which is followed by a rapid de-activation that is negatively regulated by the ABIN-2 protein.

## Acknowledgments

This work was supported by grants from the National Science Council (NRPGM92GM064 and NSC93-2752-B-010-004-PAE), and the VTY Joint Research Program, Tsou's Foundation (VTY-91-G5-04), Taiwan. We thank Dr. Jin-Yuan Su for the gift of human ABIN-2 antibody.

## References

[1] F. Mercurio, A.M. Manning, Multiple signals converging on NF-kappaB, Curr. Opin. Cell Biol. 11 (1999) 226–232.
[2] A.A. Beg, W.C. Sha, R.T. Bronson, S. Ghosh, D. Baltimore, Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B, Nature 376 (1995) 167–170.
[3] Y. Xu, S. Bialik, B.E. Jones, Y. Iimuro, R.N. Kitsis, A. Srinivasan, D.A. Brenner, M.J. Czaja, NF-kappaB inactivation converts a hepatocyte cell line TNF-alpha response from proliferation to apoptosis, Am. J. Physiol. 275 (1998) C1058–C1066.
[4] I. Kirillova, M. Chaisson, N. Fausto, Tumor necrosis factor induces DNA replication in hepatic cells through nuclear factor kappaB activation, Cell Growth Differ. 10 (1999) 819–828.
[5] M.J. FitzGerald, E.M. Webber, J.R. Donovan, N. Fausto, Rapid DNA binding by nuclear factor kappa B in hepatocytes at the start of liver regeneration, Cell Growth Differ. 6 (1995) 417–427.
[6] D.E. Cressman, L.E. Greenbaum, B.A. Haber, R. Taub, Rapid activation of post-hepatectomy factor/nuclear factor kappa B in hepatocytes, a primary response in the regenerating liver, J. Biol. Chem. 269 (1994) 30429–30435.
[7] F. Mercurio, A.M. Manning, NF-kappaB as a primary regulator of the stress response, Oncogene 18 (1999) 6163–6171.
[8] J.T. Cooper, D.M. Stroka, C. Brostjan, A. Palmetshofer, F.H. Bach, C. Ferran, A20 blocks endothelial cell activation through a NF-kappaB-dependent mechanism, J. Biol. Chem. 271 (1996) 18068–18073.
[9] M. Jaattela, H. Mouritzen, F. Elling, L. Bastholm, A20 zinc finger protein inhibits TNF and IL-1 signaling, J. Immunol. 156 (1996) 1166–1173.
[10] K. Heyninck, R. Beyaert, A20 inhibits NF-kappaB activation by dual ubiquitin-editing functions, Trends Biochem. Sci. 30 (2005) 1–4.
[11] E.G. Lee, D.L. Boone, S. Chai, S.L. Libby, M. Chien, J.P. Lodolce, A. Ma, Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice, Science 289 (2000) 2350–2354.
[12] S. Van Huffel, F. Delaei, K. Heyninck, D. De Valck, R. Beyaert, Identification of a novel A20-binding inhibitor of nuclear factor-kappa B activation termed ABIN-2, J. Biol. Chem. 276 (2001) 30216–30223.
[13] R. Beyaert, K. Heyninck, S. Van Huffel, A20 and A20-binding proteins as cellular inhibitors of nuclear factor-kappa B-dependent gene expression and apoptosis, Biochem. Pharmacol. 60 (2000) 1143–1151.
[14] W.K. Liu, P.F. Yen, C.Y. Chien, M.J. Fann, J.Y. Su, C.K. Chou, The inhibitor ABIN-2 disrupts the interaction of receptor-interacting protein with the kinase subunit IKKgamma to block activation of the transcription factor NF-kappaB and potentiate apoptosis, Biochem. J. 378 (2004) 867–876.
[15] Y. Iimuro, T. Nishiura, C. Hellerbrand, K.E. Behrns, R. Schoonhoven, J.W. Grisham, D.A. Brenner, NFkappaB prevents apoptosis and liver dysfunction during liver regeneration, J. Clin. Invest. 101 (1998) 802–811.
[16] J. Plumpe, N.P. Malek, C.T. Bock, T. Rakemann, M.P. Manns, C. Trautwein, NF-kappaB determines between apoptosis and proliferation in hepatocytes during liver regeneration, Am. J. Physiol. Gastrointest. Liver Physiol. 278 (2000) G173–G183.
[17] G.M. Higgins, R.M. Anderson, Experimental pathology of the liver, Restoration of the liver of the white rat following partial surgical removal, Arch. Pathol. 12 (1931) 186–202.
[18] B. Hogan, R. Beddington, F. Costantini, E. Lacy, Manipulating the Mouse Embryo: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1994.
[19] J. Sambrook, D.W. Russell, Molecular Cloning: A Laboratory Manual, third ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001.
[20] T.F. Tsai, K.S. Chen, J.S. Weber, M.J. Justice, A.L. Beaudet, Evidence for translational regulation of the imprinted Snurf-Snrpn locus in mice, Hum. Mol. Genet. 11 (2002) 1659–1668.

[21] E. Harlow, D. Lane, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1999.
[22] M.B. Arvelo, J.T. Cooper, C. Longo, S. Daniel, S.T. Grey, J. Mahiou, E. Czismadia, G. Abu-Jawdeh, C. Ferran, A20 protects mice from D-galactosamine/lipopolysaccharide acute toxic lethal hepatitis, Hepatology 35 (2002) 535–543.
[23] M. Tewari, P. Dobrzanski, K.L. Mohn, D.E. Cressman, J.C. Hsu, R. Bravo, R. Taub, Rapid induction in regenerating liver of RL/IF-1 (an I kappa B that inhibits NF-kappa B, RelB-p50, and c-Rel-p50) and PHF, a novel kappa B site-binding complex, Mol. Cell. Biol. 12 (1992) 2898–2908.
[24] Q. Li, D. Van Antwerp, F. Mercurio, K.F. Lee, I.M. Verma, Severe liver degeneration in mice lacking the IkappaB kinase 2 gene, Science 284 (1999) 321–325.
[25] M. Tanaka, M.E. Fuentes, K. Yamaguchi, M.H. Durnin, S.A. Dalrymple, K.L. Hardy, D.V. Goeddel, Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKK-beta-deficient mice, Immunity 10 (1999) 421–429.
[26] D. Rudolph, W.C. Yeh, A. Wakeham, B. Rudolph, D. Nallainathan, J. Potter, A.J. Elia, T.W. Mak, Severe liver degeneration and lack of NF-kappaB activation in NEMO/IKKgamma-deficient mice, Genes Dev. 14 (2000) 854–862.
[27] A. Krikos, C.D. Laherty, V.M. Dixit, Transcriptional activation of the tumor necrosis factor alpha-inducible zinc finger protein, A20, is mediated by kappa B elements, J. Biol. Chem. 267 (1992) 17971–17976.
[28] P.J. Chiao, S. Miyamoto, I.M. Verma, Autoregulation of I kappa B alpha activity, Proc. Natl. Acad. Sci. USA 91 (1994) 28–32.
[29] I.E. Wertz, K.M. O'Rourke, H. Zhou, M. Eby, L. Aravind, S. Seshagiri, P. Wu, C. Wiesmann, R. Baker, D.L. Boone, A. Ma, E.V. Koonin, V.M. Dixit, De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signaling, Nature 430 (2004) 694–699.
[30] M. Klinkenberg, S. Van Huffel, K. Heyninck, R. Beyaert, Functional redundancy of the zinc fingers of A20 for inhibition of NF-kappaB activation and protein–protein interactions, FEBS Lett. 498 (2001) 93–97.
[31] M.L. Chaisson, J.T. Brooling, W. Ladiges, S. Tsai, N. Fausto, Hepatocyte-specific inhibition of NF-kappaB leads to apoptosis after TNF treatment, but not after partial hepatectomy, J. Clin. Invest. 110 (2002) 193–202.