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# Adiponectin induces the transforming growth factor decoy receptor BAMBI in human hepatocytes

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ABSTRACT

**Transforming growth factor (TGF) β is the central cytokine in fibrotic liver diseases. We analyzed whether hepatoprotective adiponectin directly interferes with TGFβ1 signaling in primary human hepatocytes (PHH). Adiponectin induces the TGFβ decoy receptor BMP-and activin-membrane-bound inhibitor (BAMBI) in PHH. Overexpression of BAMBI in hepatoma cells impairs TGFβ-mediated phosphorylation of SMAD2 and induction of connective tissue growth factor. BAMBI is lower in human fatty liver with a higher susceptibility to liver fibrosis and negatively correlates with BMI of the donors. Hepatic BAMBI is reduced in rodent models of liver inflammation and fibrosis. In summary, the current data show that hepatoprotective effects of adiponectin include induction of BAMBI which is reduced in human fatty liver and rodent models of metabolic liver injury.**


## 1. Introduction

Non-alcoholic steatohepatitis (NASH) is the most prevalent liver disease in western countries and about 2–4% of adults are suggested to meet current diagnostic criteria for NASH [1,2]. NASH features liver fibrosis and inflammation, and a prospective study has proven that NASH progresses to liver cirrhosis in about 20% of the patients [3]. The pathophysiological events causing NASH and progression of liver fibrosis are incompletely understood, and importantly no treatment regimes have been established [4–6].

Prevalence of NASH is increased in obesity, insulin resistance and type 2 diabetes mellitus and is considered to constitute the hepatic form of the metabolic syndrome [1,7]. Obesity is accompanied by low systemic adiponectin, an adipose tissue produced protein which ameliorates insulin resistance and hepatic steatosis partly by stimulating β-oxidation [5,8]. Low adiponectin levels are closely associated with the degree of hepatic steatosis, necroinflammation and fibrosis independent of insulin resistance and body mass index (BMI) in NASH patients [9,10]. In animal models of NASH adiponectin lowers inflammation, reactive oxygen species production and liver fibrosis [5,11].

Transforming growth factor (TGF) β1 is the central cytokine in fibrotic liver diseases and induces synthesis of extracellular matrix proteins in hepatic stellate cells. In hepatocytes TGFβ1 increases synthesis of connective tissue growth factor (CTGF), and may promote hepatocyte cell death which further contributes to liver dysfunction [12–14].

Adiponectin signals through two receptors, AdipoR1 and AdipoR2, with both of them being expressed in liver cells [15,16]. Increasing AdipoR2 expression in the liver ameliorates hepatic injury in the methionine-choline deficient diet (MCD) model partly by stimulating PPARα [17]. AdipoR1 is involved in AMP activated kinase (AMPK) mediated suppression of the lipogenic transcription factor sterol regulatory element-binding protein (SREBP) 1c in hepatocytes [18]. Adiponectin inhibits hepatic stellate cell (HSC) proliferation via activation of AMPK indicating that AdipoR1 may have a role in antifibrotic effects of this adipokine [19].

TGFβ signaling is silenced by the TGFβ pseudoreceptor BMP and activin membrane-bound inhibitor (BAMBI) found to be exclusively expressed on HSC [20]. Intestinal translocation of endotoxin is enhanced in patients with liver diseases, and endotoxin-induced toll-like receptor 4 (TLR4) signaling downregulates BAMBI. Expression of a dominant negative form of BAMBI mimics fibrotic effects of TLR4 in HSC confirming a crucial role of BAMBI in inflammation mediated progression of liver fibrosis [20].

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In the current study, we demonstrate that antifibrotic effects of adiponectin include induction of BAMBI in primary human hepatocytes and hepatic stellate cells.

## 2. Materials and methods

### 2.1. Culture media and reagents

Dulbecco's modified eagle medium (DMEM) was from PAA (Karlsruhe, Germany), RNeasy Mini Kit was from Qiagen (Hilden, Germany) and oligonucleotides were synthesized by Metabion (Planegg-Martinsried, Germany). LightCycler FastStart DNA Master SYBR Green I was purchased from Roche (Mannheim, Germany). Palmitic acid, oleic acid, metformin and fenofibrate were ordered from Sigma (Deisenhofen, Germany). The AMPK inhibitor compound C and the InSolution™ NF-κB Activation Inhibitor were from Calbiochem (Darmstadt, Germany). GAPDH antibody was from New England Biolabs GmbH (Frankfurt, Germany). BAMBI antibodies were from Abnova (Heidelberg, Germany) and Abcam (Cambridge, UK). Recombinant full-length human adiponectin, and recombinant human TGFβ1 were from R&D Systems (Wiesbaden-Nordenstadt, Germany). The monoclonal CTGF antibody was from Abnova (Heidelberg, Germany). SMAD2/3, SMAD3, phospho-SMAD2 (Ser465/467) and phospho-SMAD3 (Ser423/425) antibodies were from New England Biolabs GmbH (Frankfurt, Germany).

### 2.2. Primary liver cells

Human liver tissues for cell isolation were obtained from liver resections of patients undergoing partial hepatectomy for metastatic liver tumors of colorectal cancer. Experimental procedures were performed according to the guidelines of the charitable state controlled foundation HTCR (Human Tissue and Cell Research), with the informed patient's consent approved by the local ethical committee of the University of Regensburg [21]. Primary human hepatocytes were isolated and cultivated in serum-free medium (DMEM supplemented with 4.5 g/l glucose, 0.4 ng/ml hydrocortisone, 0.415 mU/ml insulin, 2 mM glutamine, and 100 U/ml penicillin/streptomycin) for 3 days as previously described [22]. Isolation and culture of hepatic stellate cells (HSC) were performed as described [23,24]. HSCs were cultivated up to 13 days.

### 2.3. Human liver tissues

Liver tissues for immunoblot analysis were obtained of seven patients (4 females, 3 males) without and seven patients (2 females, 5 males) with biopsy proven steatosis. Anamnesis excluded alcohol intake, drugs and viral infections as cause for fatty liver disease. Surgery was done because of hepatic metastases of extrahepatic tumours and only healthy tissue was used. Here, neither inflammation nor fibrosis was detected in the livers by the pathologist. Age (58 ± 13 and 62 ± 15 years) and BMI (26.8 ± 3.7 and 29.3 ± 3.2 kg/m$^2$) were similar between controls and patients with hepatic steatosis. BMI of one control was not known. Hepatocellular carcinoma tissue and adjacent healthy tissue were obtained of eight patients. Experimental procedures were performed according to the guidelines of the charitable state controlled foundation HTCR (Human Tissue and Cell Research), with the informed patient's consent approved by the local ethical committee of the University of Regensburg [21].

### 2.4. Animal models

Male C57 Bl/6 mice were purchased from Charles River Laboratories (Sulzfeld, Germany) at 6 weeks of age and housed in a 22 °C controlled room under a 12 h light-dark cycle with free access to food and water. After acclimatization mice were divided into three groups (4 mice per group) and fed either a standard diet (3% w/w fat), the so called Paigen-diet (1.25% (w/w) cholesterol, 0.5% (w/w) cholic acid, and 15% (w/w) fat) or MCD diet for 14 weeks. All chows were prepared by Ssniff (Soest, Germany).

The Paigen-diet induced hepatic steatosis and inflammation [25,26]. Final body weight of the animals was 18.3 ± 0.5 g in the control group and 23.3 ± 1.8 g in the Paigen-diet group ($p = 0.029$). Adiponectin was 2.4 ± 0.4 μg/ml in serum of control mice and 2.1 ± 0.1 μg/ml in serum of animals fed a Paigen diet. MCD diet causes hepatic steatosis (triglycerides were significantly induced, data not shown), inflammation (significant induction of TNF mRNA, data not shown) and fibrosis (confirmed by Sirius red staining and α-smooth muscle actin immunoblot, data not shown) as has already been described [27]. Adiponectin was increased to 3.8 ± 0.5 μg/ml ($p = 0.029$ compared to control mice) and body weight was reduced to 13.8 ± 0.6 g ($p = 0.029$ compared to control mice).

All animal procedures were approved by the local committee on animal research and complied with the German Law on Animal Protection as well as the UFAW 'Handbook on the care and management of laboratory animals' 1999.

### 2.5. Monitoring of gene expression by real-time RT-PCR

Real-time RT-PCR was performed as recently described [28,29]. The primers for BAMBI were BAMBI uni: 5′-CGC CAC TCC AGC TAC ATC TT-3′ and BAMBI rev: 5′-CAG ATG TCT GTC GTG CTT GC-3′, For β-actin: β-actin uni 5′-CTA CGT CGC CCT GGA CTT CGA GC-3′, and β-actin rev: 5′-GAT GGA GCC GCC GAT CCA CAC GG-3′ were used.

### 2.6. Preparation of BAMBI expression vector

BAMBI cDNA was amplified from total RNA isolated of PHH using the oligonucleotides uni: 5′-ATG GAT CGC CAC TCC AGC TA-3′ and rev: 5′-TAC GAA TTC CAG CTT CCC GTG-3′ and cloned in the plasmid pcDNA3.1/V5-His TOPO TA (Invitrogen, Darmstadt, Germany). The insert was sequenced (Geneart, Regensburg, Germany) and plasmid DNA was transfected in HepG2 and Huh7 cells using FuGENE HD transfection reagent (Roche, Mannheim, Germany).

### 2.7. SDS–PAGE and immunoblotting

Proteins (10–20 μg) were separated by SDS–polyacrylamide gel electrophoresis and transferred to PVDF membranes (Bio-Rad, Munich, Germany). Incubations with antibodies were performed in 1.5% BSA in PBS, 0.1% Tween. Detection of the immune complexes was carried out with the ECL Western blot detection system (Amersham Pharmacia, Deisenhofen, Germany).

### 2.8. Immunohistochemistry (IHC)

Immunohistochemical studies for the expression of BAMBI utilized the EnVision+ Kit (DAKO, Glostrup, Denmark) based on a HRP labelled polymer which is conjugated with a secondary antibody. Three μm sections were cut from formalin-fixed and paraffin-embedded tissues. After deparaffinization for 15 min in Histol, tissue sections were rehydrated in descending ethanol series following antigen retrieval (microwave oven for 20 min at 800 W in sodium citrate buffer). Endogenous peroxidase activity was eliminated by subsequent incubation with 0.3% hydrogen peroxide for 10 min. After washing in TBS/0.5% Tween 20 slides were incubated for 1 h in a protein-blocking solution (DAKO). Incubation with the monoclonal BAMBI antibody (1:100-fold diluted, Abnova) was performed overnight at 4 °C in a humid chamber. After thorough washing with TBS/0.5% Tween 20, tissue sections were incubated with anti-mouse

HRP labelled polymer for 30 min. Staining was completed by incubation with DAB substrate chromogen (DAKO) according to the manufacturer's instructions.

### 2.9. Statistical analysis

Data are presented as box plots indicating median, lower and upper quartiles and range of the values. Statistical differences were analyzed by two-tailed Mann–Whitney U Test or paired Student's *t*-test, and a value of $p < 0.05$ was regarded as statistically significant. The Pearson's correlation was calculated using the PASW statistics 17.0 program.

## 3. Results

### 3.1. BAMBI is expressed in primary human hepatocytes (PHH) and hepatic stellate cells (HSC)

BAMBI has been mainly analyzed for its role in HSC because mRNA expression was not detected in hepatocytes and Kupffer cells in a recent study [20]. Purified Kupffer cells (KC) express the macrophage specific receptor CD163 but BAMBI protein was neither found in KC nor primary human monocytes (Fig. 1A). BAMBI protein was detected in liver lysate and purified PHH as a 38 kDa protein by the use of two different anti BAMBI antibodies (Fig. 1A and B and data not shown). In HSC, BAMBI was about 3 kDa smaller than in hepatocytes suggesting different posttranslational modifications (Fig. 1B). BAMBI protein was about 12-fold higher expressed in PHH compared to HSC (Fig. 1C). Real-time RT-PCR analysis revealed higher BAMBI mRNA expression in HSC compared to PHH ($p = 0.036$, data not shown). BAMBI cDNA amplified from reverse transcribed RNA of PHH accorded to the published sequence (NM_012342) further confirming BAMBI expression in hepatocytes (data not shown). Immunohistochemistry using healthy liver tissue confirmed high expression of BAMBI in hepatocytes. BAMBI was also detected in endothelial cells of blood vessels and was found to be weakly expressed in cholangiocytes but was not detected in Kupffer cells (Fig. 1D–F).

### 3.2. Adiponectin induces BAMBI in PHH and HSC

Adiponectin mediated upregulation of BAMBI mRNA in PHH was demonstrated by real-time RT-PCR (Fig. 2A). Furthermore, adiponectin significantly induced BAMBI protein in PHH (Fig. 2B and C) and HSC (Fig. 2D and E). TGFβ1 may impair adiponectin activity, and it was analysed whether adiponectin still induces BAMBI in the presence of TGFβ1. BAMBI was similarly induced by adiponectin in control and TGFβ1 incubated PHH, and was not regulated by TGFβ1 (Fig. 2F). Furthermore, adiponectin preincubation prevented TGFβ1-mediated upregulation of its target protein connective tissue growth factor (CTGF). Interestingly adiponectin also lowered CTGF in PHHs not incubated with TGFβ1 (Fig. 2F and G).

### 3.3. NF-κB Activation Inhibitor partly blocks induction of BAMBI by adiponectin and metformin induces BAMBI in PHHs

The main downstream effectors of adiponectin are PPARα and AMPK [16]. Stimulation of PHH with the PPARα agonist fenofibrate (0.5 and 1 mM for 24 h) did not affect BAMBI protein (data not shown). Metformin at a concentration of 0.5 and 1 mM activated AMPK (data not shown) and induced BAMBI in PHH (Fig. 2H). However, blockage of AMPK by its antagonist compound C only tended to reduce induction of BAMBI by adiponectin but this effect was not significant when analysed in PHHs of three different donors (Fig. 2H and data not shown). Activation of NF-κB by adiponectin has also been demonstrated and InSolution™ NF-κB Activation Inhibitor reduced adiponectin mediated upregulation of BAMBI by about 50% ($p = 0.03$ when three independent experiments were analysed) (Fig. 2I).

### 3.4. BAMBI impairs TGFβ1 mediated induction of CTGF

Despite expression of BAMBI mRNA BAMBI protein was hardly detectable in hepatoma cell lines HepG2, Hep3B and Huh7 (Fig. 3A and B). To prove that BAMBI antagonizes TGFβ1 activity in hepatoma cells which are commonly used as a model to study hepatocyte function [30,31] BAMBI protein was transiently overexpressed in HepG2 cells, and cells were subsequently treated with increasing amounts of TGFβ1 for 48 h. CTGF is a well described TGFβ1 induced protein [32] and was analyzed in control- and BAMBI-transfected HepG2 cells. Whereas CTGF was markedly induced by 1, 3 and 5 ng/ml TGFβ1 in control transfected cells this upregulation was significantly impaired in BAMBI-expressing HepG2 cells (Fig. 3C and D). When BAMBI was overexpressed in HepG2 or Huh7 cells which

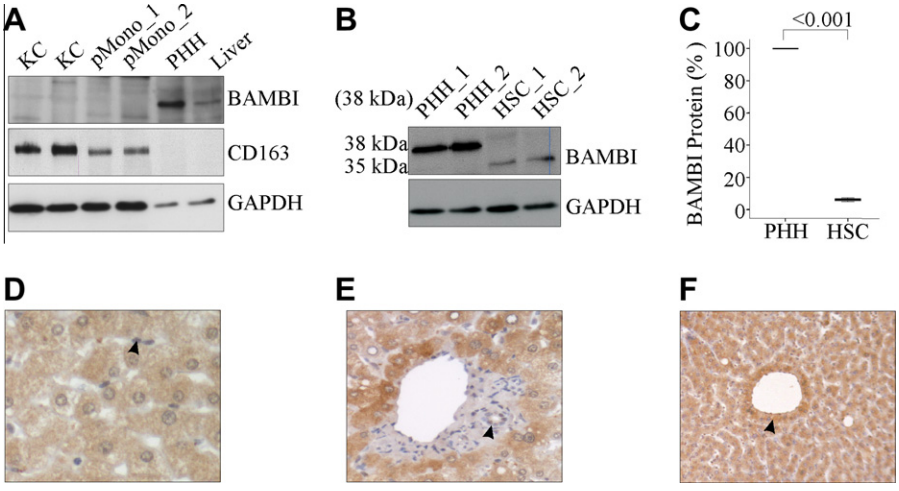


**Fig. 1.** BAMBI protein is expressed in hepatocytes and hepatic stellate cells. (A) BAMBI and CD163 protein in primary human Kupffer cells (KC), primary human monocytes (pMono), PHH and total liver lysate. (B) BAMBI in PHH and HSC of two different donors. (C) Quantification of the immunoblots including data of 4 independent experiments partly shown in B. (D) BAMBI protein was analysed by immunohistochemistry in healthy liver tissue and is strongly expressed in hepatocytes but not Kupffer cells (arrow). (E) BAMBI is weakly expressed by cholangiocytes (arrow). (F) BAMBI is expressed by endothelial cells (arrow).

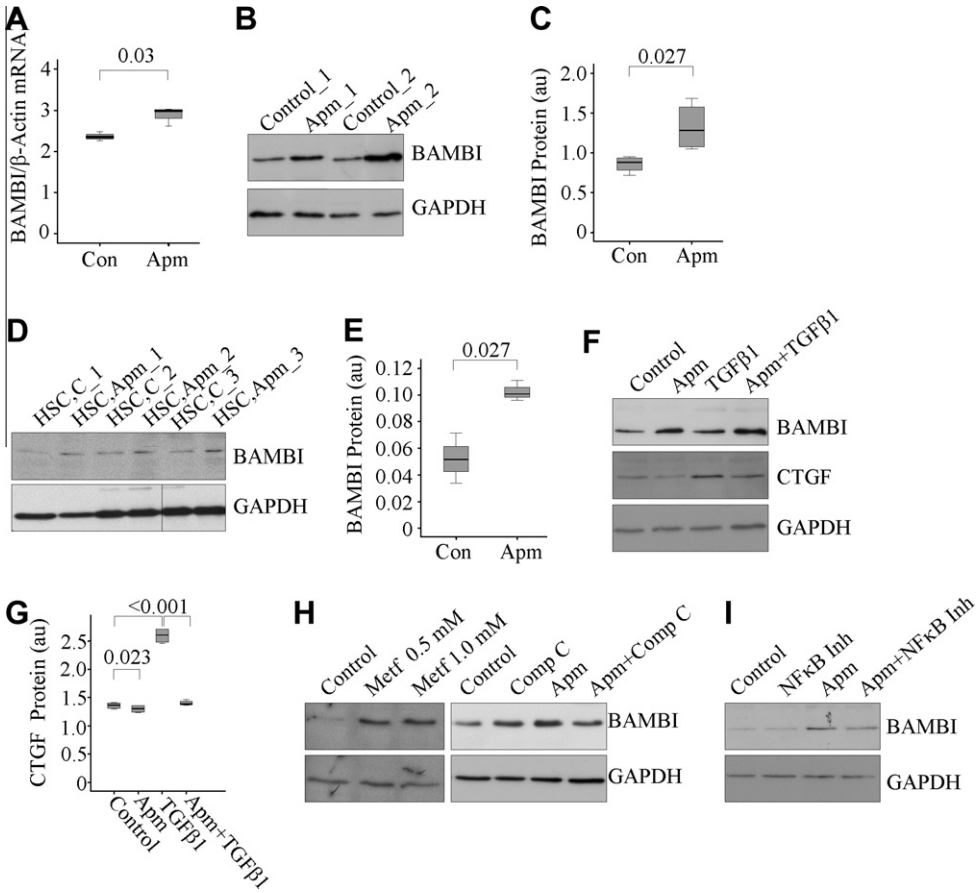


**Fig. 2.** Adiponectin upregulates BAMBI in primary human hepatocytes (PHH) and hepatic stellate cells (HSC). (A) BAMBI mRNA in PHH treated with PBS as solvent control (Con) or 10 μg/ml adiponectin (Apm). Data of 4 independent experiments were calculated. (B) PHH of two donors were incubated with PBS as solvent control or 10 μg/ml adiponectin (Apm) for 24 h, and BAMBI and GAPDH were determined by immunoblot. (C) Quantification of the immunoblots of 4 independent experiments partly shown in B (arbitray units, au). (D) Primary human hepatic stellate cells of 3 donors were incubated with PBS as solvent control or 10 μg/ml adiponectin (Apm) for 24 h and BAMBI and GAPDH were determined by immunoblot. (E) Quantification of the immunoblot shown in D (arbitray units, au). (F) BAMBI and CTGF in PHH incubated with PBS as solvent control or adiponectin for 24 h and in PHHs treated with TGFβ1 (4 ng/ml) for 24 h with or without preincubation with adiponectin for 1 h. (G) Quantification of CTGF protein of 3 independent experiments partly shown in (F). (H) BAMBI in PHH incubated with metformin (Metf) or solvent as control for 24 h and in cell lysates of PHH treated with adiponectin, solvent as control, compound C or compound C and adiponectin for 24 h. (I) BAMBI in cell lysates of PHH treated with adiponectin, solvent as control, 0.5 μM InSolution™ NF-κB Activation Inhibitor or 0.5 μM InSolution™ NF-κB Activation Inhibitor and adiponectin for 24 h.

were stimulated with 1 ng/ml TGFβ1 for 30 min phosphorylation of SMAD2 was significantly reduced suggesting that BAMBI impairs early steps in TGFβ1 signal transduction pathways (Fig. 3E and F and data not shown). Phosphorylation of SMAD3, however, was similar in control- and BAMBI transfected cells (Fig. 3E and data not shown).

Low BAMBI protein in hepatocellular carcinoma cell lines led us to analyse BAMBI in hepatocellular carcinoma and adjacent non-tumorous tissue of eight patients and BAMBI protein was significantly higher in the latter (Fig. 3G and data not shown). Adiponectin did not induce BAMBI in HepG2 cells (data not shown).

### 3.5. BAMBI in human fatty liver

Liver tissue was obtained of patients with and without biopsy proven hepatic steatosis with no histological signs of inflammation or fibrosis. Immunoblot analysis revealed that BAMBI protein was significantly reduced in steatotic liver (Fig. 4A and B). Incubation of PHHs with either 0.3 mM palmitic acid or oleic acid did not reduce BAMBI protein excluding hepatic lipid accumulation as reason for low BAMBI in fatty liver (data not shown). Liver BAMBI protein negatively correlated with BMI of the patients ($r = -0.702$, Fig. 4C) indicating that factors associated with obesity like low adiponectin may contribute to reduced hepatic BAMBI protein.

### 3.6. BAMBI in rodent models of liver injury

Systemic and liver inflammation which partly depend on TLR4 in concert with steatosis have been described in mice kept on a Paigen diet, and BAMBI was markedly reduced in the liver of mice kept on this chow (Fig. 4D and E). The methionine-choline deficient diet fed mouse is used as a NASH model and BAMBI was also significantly lower in the liver of these animals (Fig. 4F and G).

## 4. Discussion

In the current study, BAMBI protein is found highly expressed in PHH. This has been confirmed by the use of two different BAMBI antibodies for immunoblot analysis, immunohistochemistry and amplification of BAMBI cDNA from hepatocyte RNA by different primer pairs. Although HSC express BAMBI mRNA at higher concentrations than PHHs BAMBI protein is hardly detectable by immunoblot analysis in these cells. This indicates that BAMBI protein levels may be regulated by posttranscriptional/posttranslational mechanisms. BAMBI mRNA is also highly expressed in hepatoma cells whereas protein levels are low in these cell lines and in HCC tissues. Therefore, at least in liver cells BAMBI mRNA does not necessarily predict protein levels. In endothelial cells, BAMBI protein concentrations are predominantly controlled by

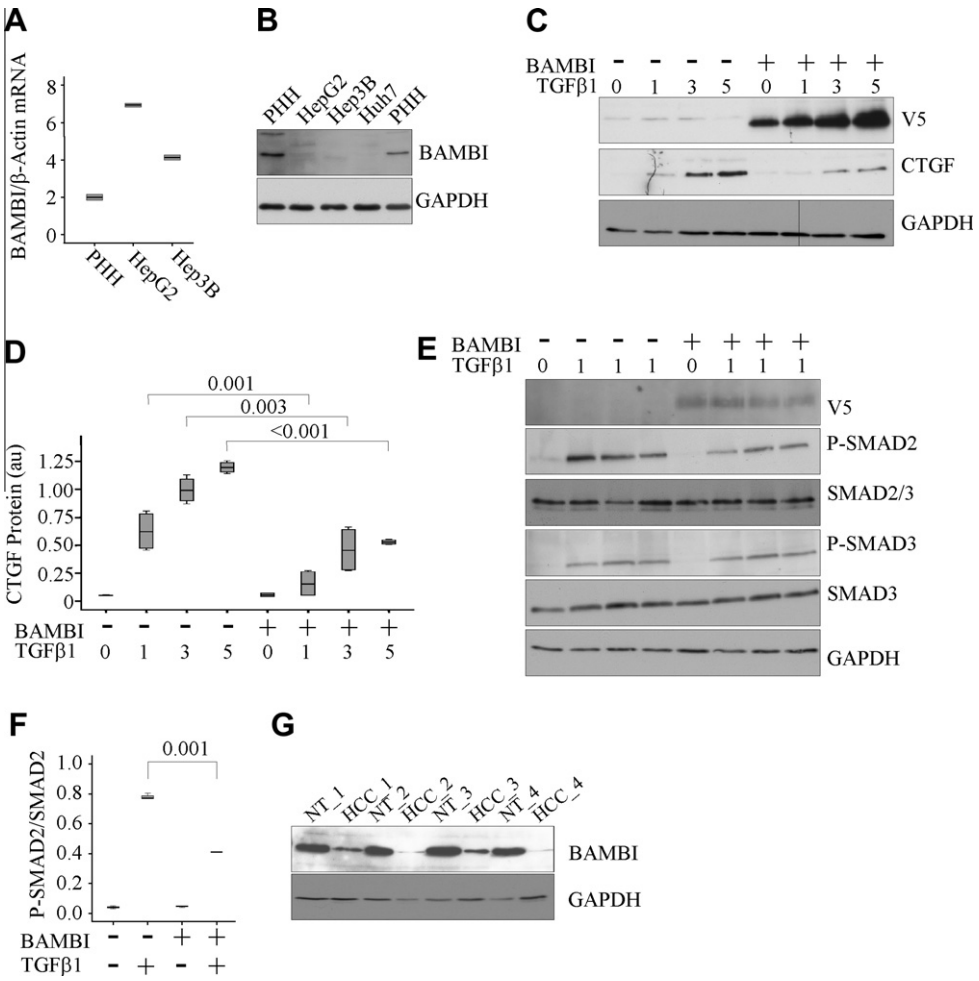


**Fig. 3.** BAMBI impairs TGFβ1 activity in hepatoma cells (A) BAMBI mRNA in primary human hepatocytes (PHH), HepG2 and Hep3B cells. (B) BAMBI protein in PHH, HepG2, Hep3B and Huh7 cells. (C) HepG2 cells expressing BAMBI fused to a V5 tag and control transfected cells were incubated with TGFβ1 (1, 3 and 5 ng/ml) for 48 h and CTGF was analyzed in the cell lysates. (D) Quantification of the immunoblots of 3 independent experiments with a representative result shown in C (arbitray units, au). (E) Huh7 cells expressing BAMBI fused to a V5 tag and control transfected cells were incubated with TGFβ1 (1 ng/ml) for 30 min and P-SMAD2, SMAD2/3, P-SMAD3 and SMAD3 were analyzed in the cell lysates. (F) Quantification of the P-SMAD2/SMAD2 ratio from the immunoblot data of 3 independent experiments shown in (E). (G) BAMBI protein in hepatocellular carcinoma and adjacent healthy tissues (NT) of 4 patients.

lysosomal and auto lysosomal degradation [33] and similar regulatory pathways may exist in liver cells and contribute to contradictory findings for mRNA and protein levels.

BAMBI protein is also detected in endothelial cells of blood vessels as has been already shown [33] and cholangiocytes. Kupffer cells do not produce BAMBI protein in accordance with published mRNA expression data [20].

Predicted molecular weight of BAMBI is 26 kDa and the BAMBI protein detected in hepatocytes has a molecular weight of 38 kDa, in HSC BAMBI is a 35 kDa protein indicating posttranslational modification of BAMBI that differs in PHH and HSC. BAMBI protein in human and rodent liver resembles the 38 kDa protein although species-specific differences of BAMBI protein have been described in the kidney where the mouse protein has a molecular weight of about 27 kDa and the human protein of about 29 kDa. The 35 kDa BAMBI isoform expressed by human HSC is not detected by immunoblot analysis in whole liver lysate of mice and humans. This finding is in line with low abundance of BAMBI protein in HSC which, furthermore, only make up 5% of total liver cells.

BAMBI protein is significantly reduced in human fatty liver, and here negatively correlates with BMI of the patients suggesting a regulatory role of adipokines or cytokines which are correlated with BMI. BMI has been identified as an independent risk factor for fibrosis in NASH and low liver BAMBI may be one factor explaining this association [34]. Systemic adiponectin is inversely related to BMI [35], and BAMBI is induced by physiological amounts of adiponectin in hepatocytes suggesting that reduced hepatic BAMBI in human fatty liver is in part a consequence of low systemic adiponectin.

BAMBI is even more prominently suppressed in rodent NASH and rodent liver fibrosis. Systemic adiponectin is significantly reduced in NASH whereas systemic levels of endotoxin are increased [5,36]. Lipopolysaccharide (LPS) downregulates BAMBI mRNA in HSC [20] but whether it lowers BAMBI protein in these cells or PHHs has not been studied so far. Nevertheless, low BAMBI in NASH liver may contribute to enhanced TGFβ signaling and subsequently to progressive fibrosis.

TGFβ is well known to upregulate CTGF [13,37] and overexpression of BAMBI in hepatoma cells impairs CTGF induction. Adiponectin also induces BAMBI in the presence of TGFβ and blocks CTGF upregulation. Interestingly, adiponectin modestly reduces CTGF in PHHs independent of TGFβ and further studies have to reveal the pathways involved herein. HSC are the main producers of extracellular matrix in the fibrotic liver [12] and adiponectin has

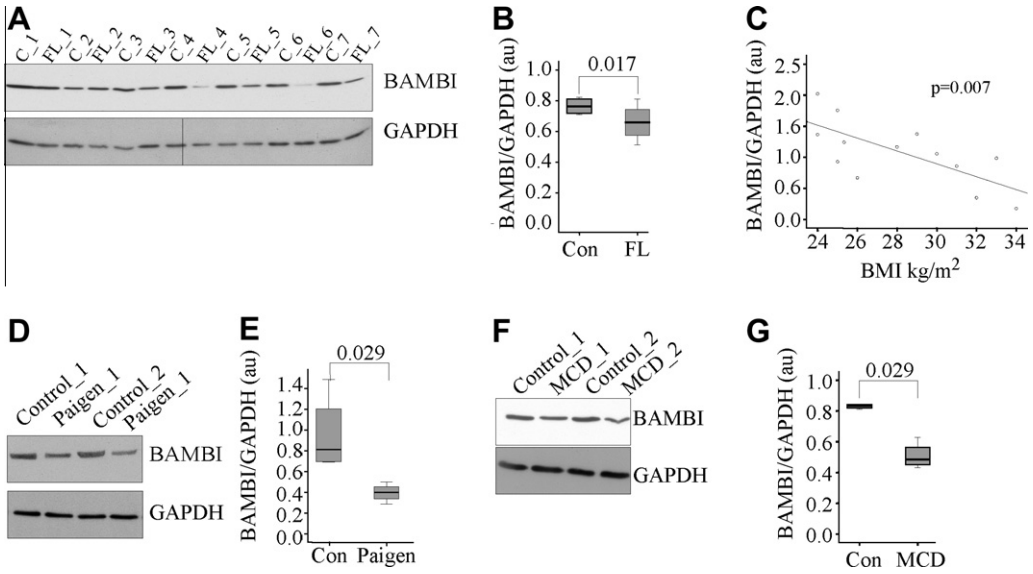


**Fig. 4.** BAMBI in human fatty liver and rodent models of liver injury. (A) BAMBI and GAPDH were analyzed in liver tissues of 7 patients without (C_1 to C_7) and 7 patients with histological defined hepatic steatosis (FL_1 to FL_7). (B) Quantification of the BAMBI immunoblot shown in B (arbitray units, au). (C) Correlation of BMI and liver BAMBI protein. (D) BAMBI in the liver of mice fed a Paigen diet or control chow. (E) Quantification of the immunoblots partly shown in (E) (arbitray units, au). (F) BAMBI in the liver of mice fed a MCD diet or control chow. (G) Quantification of the immunoblots partly shown in G (arbitray units, au).

been shown to attenuate TGFβ mediated CTGF expression [38]. Adiponectin upregulates BAMBI in HSC and this may partly contribute to the lower synthesis of CTGF described.

BAMBI has been shown to interfere with complex formation of TGFβ type I and II receptors, and furthermore, inhibits binding of SMAD3 to the TGFβ type I receptor thereby blocking SMAD3 phosphorylation [39,40]. In HeLa and HEK293T overexpressing BAMBI TGFβ-mediated phosphorylation of SMAD2 and -3 is impaired [40]. In the hepatoma cell lines studied herein BAMBI only lowers phosphorylation of SMAD2 whereas activation of SMAD3 is not affected. SMAD2 and SMAD3 have distinct functions in TGFβ signaling and selective activation of single SMADs has been described [41]. Transcriptional activation of the CTGF promoter is mediated via activated SMAD2 but not SMAD3 [32] suggesting that BAMBI associated inhibition of SMAD2 phosphorylation most likely contributes to impaired induction of CTGF. However, the precise mechanisms leading to lower SMAD2 activation by BAMBI has to be evaluated in further studies.

Downstream effectors of adiponectin are AMPK and PPARα [16]. Fenofibrate which is a PPARα agonist has no effect on BAMBI protein in PHHs. AMPK is a downstream effector of adiponectin and metformin but inhibition of this kinase by compound C does not efficiently block adiponectin-mediated upregulation of BAMBI. Therefore, further studies using more specific approaches like shRNA mediated suppression of AMPK subunits or dominant negative acting AMPK isoforms are needed to clarify the role of this kinase in the upregulation of BAMBI. Furthermore, additional kinases like p38 MAPK which is activated by adiponectin and metformin [24,42] may be involved. Adiponectin activates NF-κB [24,43,44] and inhibition of NF-κB partly blocks adiponectin-mediated upregulation of BAMBI suggesting that this pathway is involved. LPS activates NF-κB and suppresses BAMBI in hepatic stellate cells whereas BAMBI mRNA is not altered by LPS in murine mesangial cells and human umbilical vein endothelial cells [20,33]. These data suggest cell type specific regulation of BAMBI by LPS.

Nevertheless, metformin induces BAMBI in PHHs providing a possible mechanism for its already described antifibrotic effects [45]. In mouse cardiac fibroblasts metformin inhibits TGFβ1-SMAD3 signaling whereas SMAD2 phosphorylation is not affected [45] suggesting that BAMBI may even have distinct functions in different cell types.

In summary, the current study shows that adiponectin interferes with TGFβ1 signaling in hepatocytes by upregulation of BAMBI which impairs activation of SMAD2 at least in hepatoma cells.

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