

# BMP and activin receptor membrane bound inhibitor: BAMBI has multiple roles in gene expression and diseases (Review)

XIAOCHANG CHEN<sup>1,2</sup>, JUE LI<sup>1</sup>, AOQI XIANG<sup>1</sup>, HUA GUAN<sup>1</sup>, PEIHONG SU<sup>1</sup>,  
LUSHA ZHANG<sup>1</sup>, DIAN ZHANG<sup>2</sup> and QI YU<sup>1</sup>

<sup>1</sup>Shaanxi Key Laboratory of Ischemic Cardiovascular Diseases, Institute of Basic and Translational Medicine;

<sup>2</sup>Department of Basic Medicine, Xi'an Medical University, Xi'an, Shaanxi 710021, P.R. China

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**Abstract.** BMP and activin membrane-bound inhibitor (BAMBI) is a transmembrane glycoprotein, known as a pseudo-receptor for TGF $\beta$ , as, while its extracellular domain is similar to that of type I TGF $\beta$  receptors, its intracellular structure is shorter and lacks a serine/threonine phosphokinase signaling motif. BAMBI can regulate numerous biological phenomena, including glucose and lipid metabolism, inflammatory responses, and cell proliferation and differentiation. Furthermore, abnormal expression of BAMBI at the mRNA and protein levels contributes to various human pathologies, including obesity and cancer. In the present review, the structure of BAMBI is briefly introduced and its associated signaling pathways and physiological functions are described. Understanding of BAMBI structure and function may contribute to knowledge regarding the occurrence of diseases, including obesity and diabetes, among others. The present review provides a theoretical foundation for the development of BAMBI as a potential biomarker or therapeutic target.

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## 1. Introduction

BMP and activin receptor membrane-bound inhibitor (*BAMBI*) was discovered by Onichtchouk *et al* (1) in 1999, and was considered a homologue of human non-metastatic gene A due to the strong structural similarity between the two molecules (2). *BAMBI* expression is highly conserved in chordates, from fish to humans; however, its expression patterns vary significantly among different animals. For example, the *BAMBI* gene is highly expressed in human kidney medulla, placenta and spleen tissues, but not in lung or muscle tissues (2), whereas in mice, *Bambi* is primarily expressed in heart, lung and testis tissues (3). BAMBI has a broad spectrum of effects, including effects on lipid metabolism through inhibition of adipocyte lipid deposition (4), on myogenesis through promotion of muscle stem cell proliferation and differentiation (5), on ovarian function through regulation of steroidogenesis and follicle-stimulating hormone (FSH) expression levels in porcine granulocytes (6), on inflammation through inhibition or modulation of inflammatory processes (7) and on tumor development through inhibition of tumor cell motility, invasion and survival (8). Given its important roles in physiological and pathological conditions, BAMBI has increasingly become the focus of research over the past two decades (Fig. 1).

## 2. Structural characteristics of BAMBI

*BAMBI* is a transmembrane glycoprotein comprising 260 amino acids with an N-terminal extracellular domain and a short C-terminal intracellular domain (9,10). Furthermore, *BAMBI* contains numerous important post-translational modification sites, including two protein kinase C phosphorylation sites, six casein kinase phosphorylation sites, three cAMP protein kinase sites and three N-acylation sites (11,12). The structure of the extracellular ligand-binding domain of *BAMBI* is similar to that of transforming growth factor  $\beta$  receptor 1 (TGF $\beta$ RI)/bone morphogenetic protein receptor type 1 (BMPRI), while *BAMBI* lacks an equivalent intracellular serine/threonine kinase structural domain (9). Therefore, *BAMBI* readily forms heterodimers with TGF $\beta$ RI/BMPRI, which can interfere with TGF $\beta$  or BMP pathways (10). Thus, BAMBI is considered a pseudo-receptor in the TGF $\beta$  and BMP signaling pathways (1,3). During binding of TGF $\beta$  family

*Correspondence to:* Professor Qi Yu, Shaanxi Key Laboratory of Ischemic Cardiovascular Diseases, Institute of Basic and Translational Medicine, 1 Xinwang Road, Weiyang, Xi'an, Shaanxi 710021, P.R. China  
E-mail: qiyu6028@hotmail.com

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members to their receptors, BAMBI can compete with type I TGF $\beta$  receptors for binding to type II TGF $\beta$  receptors. Since the serine/threonine kinase structural domain is not present in *BAMBI*, amino acid phosphorylation does not occur, thus blocking TGF $\beta$  signaling pathway transduction (Fig. 2).

### 3. Role of BAMBI in signal transduction

**TGF $\beta$  signaling.** *BAMBI*, a pseudo-receptor for TGF $\beta$ , inhibits TGF $\beta$  signaling pathway transduction, and this inhibition is mainly associated with the SMAD family molecules (13). Guillot *et al* (14) demonstrated that deletion of *BAMBI* enhances phosphorylation of the TGF $\beta$  downstream proteins, *SMAD1/5* and *ERK1/2*, thereby delineating a physiological role for *BAMBI* in endothelial environmental homeostasis and angiogenesis regulation. In addition, *BAMBI* can form a ternary complex with *SMAD7* and the TGF $\beta$  type I receptor, *ALK5/TGFBRI*, thus inhibiting the interaction between *ALK5/TGFBRI* and *SMAD3*, and ultimately affecting *SMAD3* activation (15). Meanwhile, *BAMBI* and *SMAD7* can inhibit *SMAD2* phosphorylation, and decreased levels of *Smad2* phosphorylation are associated with gastric cancer invasion (15-17). In addition, natural upregulation of *BAMBI* and *SMAD7* expression affects the prognosis of patients with acute myeloid leukemia (AML) (18). Hence, data published to date demonstrate that *BAMBI* can affect gastric cancer invasion, as well as serving as a novel biomarker for predicting prognosis in patients with AML. Notably, although *BAMBI* can inhibit TGF $\beta$  signaling, TGF $\beta$  can directly bind to the *BAMBI* transcriptional promoter through *SMAD3* and *SMAD4* to regulate *BAMBI* transcription, and *SMAD3* and *SAMD4* can synergistically enhance its transcription (19) (Fig. 3, center panel).

**Toll-like receptor 4 (TLR4) signaling.** TLRs are a class of pattern recognition receptors that are mainly expressed on the surface of innate immune cells (20). In 1997, the first mammalian TLR, *TLR4*, was discovered in human monocytes (21). When *TLR4* is activated, two signaling pathways are induced (22): The myeloid differentiation factor 88 (*MyD88*)-dependent pathway and the TIR domain bridging protein-dependent pathway. The two pathways require the NF- $\kappa$ B signaling pathway, which is a physiological regulator of the transcription and secretion of pro-inflammatory factors (23) (Fig. 3, left panel).

Lipopolysaccharide (LPS) downregulates *BAMBI* through *MyD88*/NF- $\kappa$ B-induced signaling, which in turn enhances TGF $\beta$  signaling, thereby reducing liver fibrosis in *MyD88*-deficient mice (24). It has been shown that the expression level of *BAMBI* in the livers of patients with hepatitis is significantly lower than in the livers of healthy individuals. Liu *et al* (25) found that LPS and tumor necrosis factor- $\alpha$  could further induce NF- $\kappa$ B p50-histone deacetylase 1 interaction in hepatic stellate cells (HSCs) to inhibit *BAMBI* transcription and ultimately enhance the TGF $\beta$  signaling pathway. In addition, LPS promotes miR-942 expression via NF- $\kappa$ B p50, thereby inhibiting *BAMBI* expression at the post-transcriptional level (26). He *et al* (27) found that the *TLR4* inhibitor, clio-095, could eliminate LPS-induced *BAMBI* downregulation, suggesting that activation of the *LPS/TLR4* axis may downregulate *BAMBI* expression at the mRNA and protein

levels. Wanninger *et al* (28) found that inhibitors of NF- $\kappa$ B activation partially inhibited metformin- and lipocalin-mediated upregulation of *BAMBI* in human hepatocytes. In another study, it was confirmed, by meta-analysis in publicly available hepatocellular carcinoma data cohorts, that natural *BAMBI* overexpression was present in 78% of patients with HCC (n=803), and that it was also present and upregulated in cirrhotic samples and the tumor stroma. Furthermore, upregulated *BAMBI* expression was also confirmed in *MDR2*-KO mice (29). All these results suggest that the rise and fall of *BAMBI* expression levels are inextricably linked to the development of liver diseases.

LPS is also able to downregulate the expression level of *BAMBI* (30,31), and at the same time, the activation of bacterial autophagy is correlated with the LPS-mediated decrease in *BAMBI* expression, revealing a correlation between autophagy induced by bacterial infection and the expression level of *BAMBI*. This effect needs to be realized by the activation of the *LPS/TLR4* axis (32). *BAMBI* may therefore be able to act as a biomarker of bacterial infection.

**Wnt/ $\beta$ -catenin signaling.** The Wnt/ $\beta$ -catenin pathway is a focus of intense research in the field of Wnt signaling. In humans, Wnt ligands comprise a large family of 19 glycoproteins (33). When Wnt signaling is activated, Wnt ligands first bind to the frizzled class receptor (FZD) structural domain, including the extracellular N-terminal cysteine-rich structural region of the Wnt binding domain and a single transmembrane co-receptor [low-density lipoprotein receptor-related protein 5/6 (*LRP5/LRP6*)], to form the FZD-LRP receptor complex (34,35). Subsequently, *LRP6* is phosphorylated and recruits axin to the cytoplasmic tail of *LRP6* (36,37). Next, *LRP6* interacts with axin in the presence of scattered proteins [dishevelled protein (DVL)] (38,39), thus preventing  $\beta$ -catenin phosphorylation and proteasome degradation. Finally,  $\beta$ -catenin accumulates in the cytoplasm and translocates to the nucleus, thereby activating transcription of a series of Wnt signaling target genes (40) (Fig. 3, right panel).

*BAMBI* is highly expressed in melanoma tissues and may activate the Wnt signaling pathway by negatively regulating miR-708, thus accelerating melanoma development (41). In gastric cancer cells, *BAMBI* downregulation blocks translocation of  $\beta$ -catenin from the cytoplasm to the nucleus, thus interfering with Wnt signaling (42). Proper cellular trophoblast invasion is a prerequisite for normal pregnancy. Inadequate human trophoblast invasion leads to abnormal placental development, resulting in a variety of pregnancy-related complications such as preeclampsia and intrauterine growth restriction, all of which are detrimental to the health of both the mother and the fetus (43). Zhao *et al* (44) found that BMP2 treatment increased *BAMBI* mRNA levels and activated Wnt signaling in human trophoblast cells, including increasing levels of phosphorylated *GSK3 $\beta$*  and upregulating unphosphorylated  $\beta$ -catenin and downstream cytosolic cyclin D1 levels, suggesting that the upregulation of *BAMBI* expression levels promotes human trophoblast cell invasion and thus embryo development.

Numerous studies have linked altered Wnt signaling to tumorigenesis (e.g., rectal cancer and osteosarcoma), and *BAMBI* also plays an important role in tumorigenesis caused

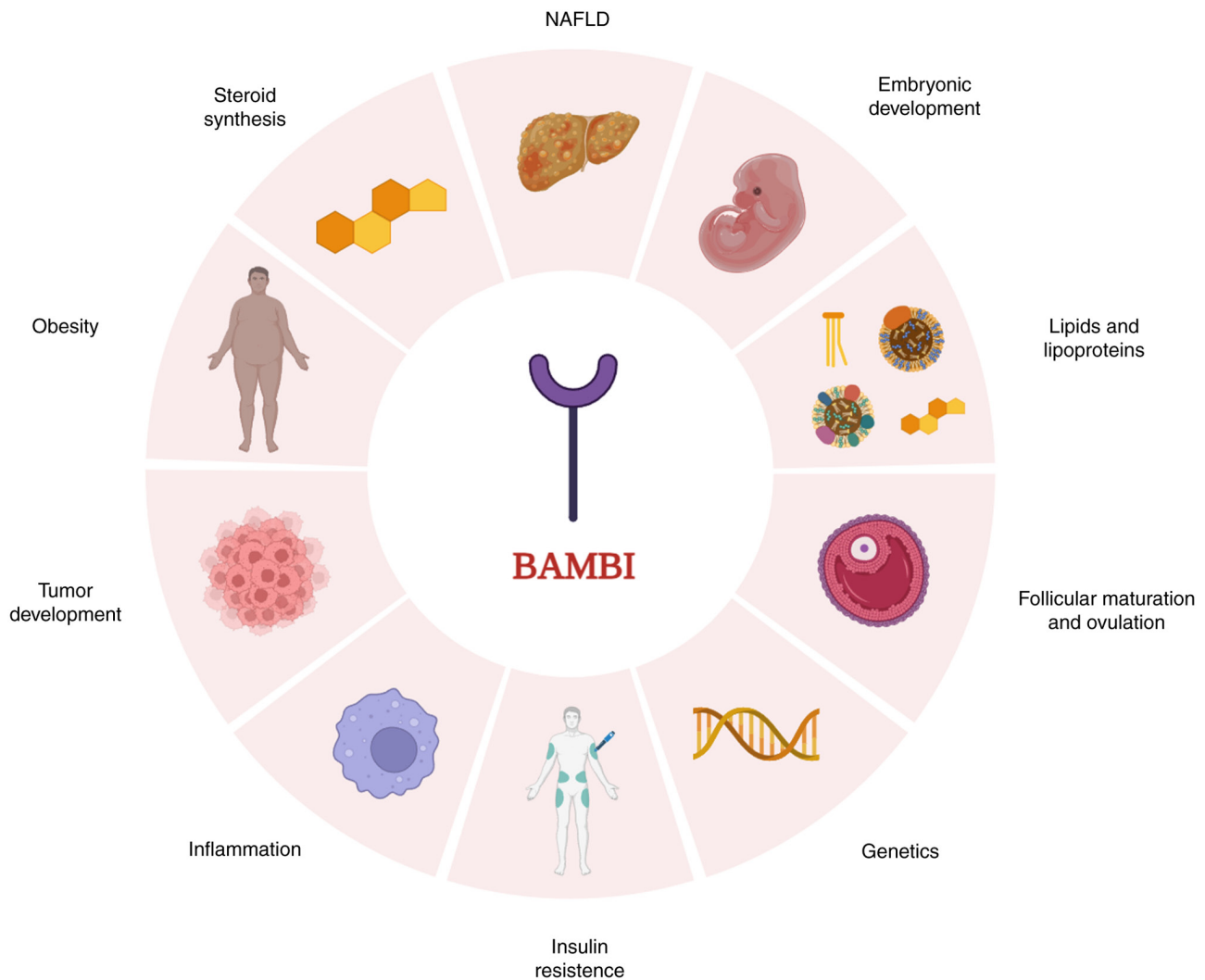


Figure 1. Diverse physiological functions of BAMBI. BAMBI is involved in a wide range of pathological processes, including inflammation, oxidative stress and adipogenesis, in diverse organs. BAMBI, BMP and activin receptor membrane-bound inhibitor; NAFLD, non-alcoholic fatty liver disease.



Figure 2. Schematic of BAMBI structure. BAMBI is a 261-amino acid transmembrane protein involved in the regulation of the TGF $\beta$  and Wnt signaling pathways. The structure of the extracellular ligand-binding domain of BAMBI is similar to that of TGF $\beta$ RI/BMPRI, whereas BAMBI lacks an equivalent intracellular Ser/Thr kinase domain. From left to right: red box, signal sequence; white box, seven conserved cysteines (vertical lines) and the putative extracellular domain of the cysteine box (gray); black, transmembrane domain; green and yellow, GS and Ser/Thr kinase domains, respectively, of TGFBR1/BMPRI; blue, intracellular domain of BAMBI. TGF $\beta$ , transforming growth factor  $\beta$ ; BAMBI, BMP and activin receptor membrane-bound inhibitor; BMPRI, bone morphogenetic protein receptor type 1; GS, glycine- and serine-rich sequence; Ser/Thr, serine/threonine.

by aberrant Wnt signaling activation. Fritzmann *et al* (45) found that *BAMBI* regulated metastasis in rectal cancer by linking the canonical Wnt pathway, and that Wnt/ $\beta$ -catenin was further activated by coactivators in the nucleus to regulate *BAMBI* expression. Sekiya *et al* (46) demonstrated

that *BAMBI* expression levels in colorectal tumor cell lines were inhibited by dominant-negative mutants of T-cell factor 4 or inhibitors of  $\beta$ -catenin-TCF interactions. Subramaniam *et al* (47) demonstrated that *BAMBI* promotes cell proliferation and survival through the Wnt/ $\beta$ -catenin pathway in HSCs, as well as other cell lines. Zhou *et al* (48) found that *BAMBI* overexpression in human osteosarcoma cells strongly induced the transcription of catenin and Wnt-induced target genes, including cyclin D1 and cell cycle protein-dependent kinases, and that endogenous *BAMBI* knockdown by siRNA blocked the Wnt pathway. Therefore, it is evident from the aforementioned results that *BAMBI* is involved in the development of the aforementioned diseases through the Wnt signaling pathway, and targeted intervention of *BAMBI* and related factors of the Wnt signaling pathway may be a novel approach for intervention or treatment of these diseases; however, further basic and clinical experiments are needed for specific application.

Overall, *BAMBI* is upregulated by Wnt signaling and enhances the activity of this pathway; *BAMBI* overexpression upregulates levels of Wnt target genes, while silencing or deletion of *BAMBI* has the opposite effect.



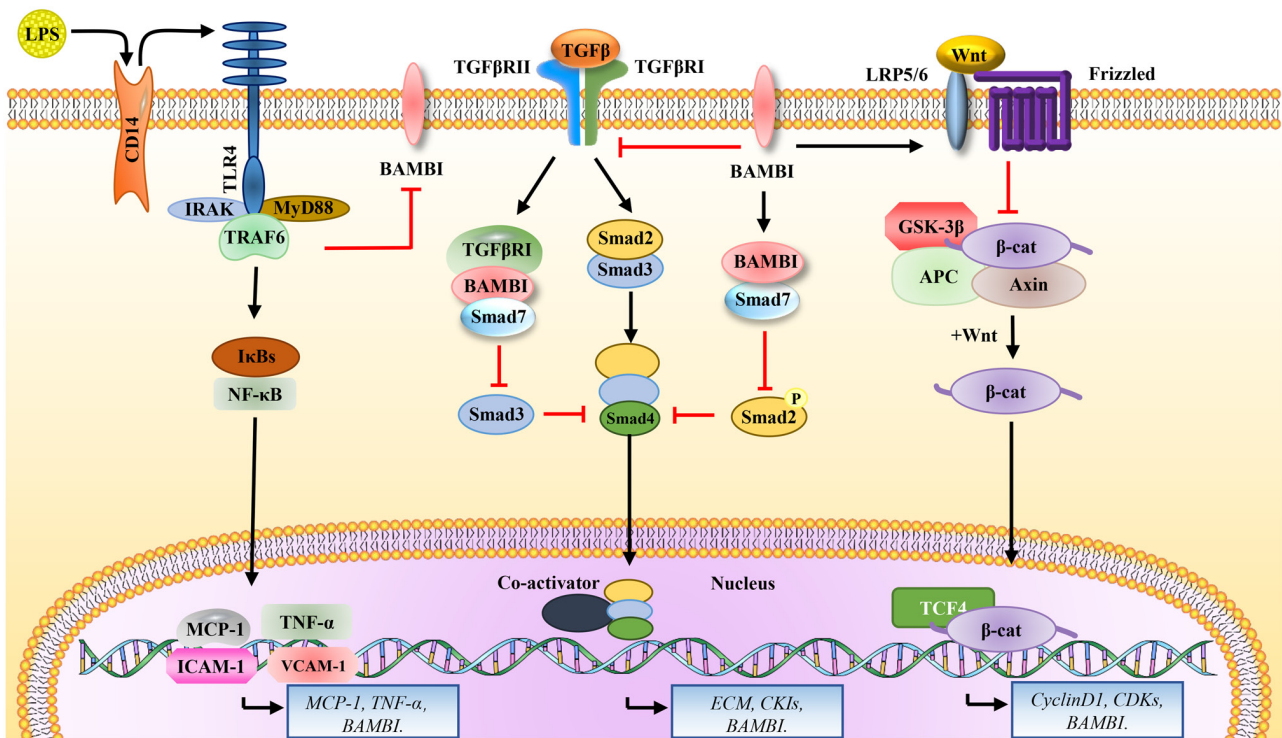


Figure 3. Signaling pathways regulated by BAMBI. BAMBI binds and affects three receptors on the cell membrane and activates downstream signaling pathways: i) LPS binds to the membrane receptor, CD14, to activate TLR4 signaling, and also downregulates BAMBI through MyD88-NF-κB-induced signaling, which in turn enhances TGFβ signaling; ii) BAMBI inhibits TGFβ signaling transduction, and this inhibition is mainly associated with the SMAD family; iii) BAMBI activates the Wnt pathway by increasing GSK3β phosphorylation and upregulating levels of unphosphorylated β-catenin and downstream cyclin D1. LPS, lipopolysaccharide; CD14, cluster of differentiation 14; TLR4, toll-like receptor 4; IRAK, interleukin 1 receptor associated kinase 1; MyD88, myeloid differentiation primary response 88; IκB, inhibitor of nuclear factor κ B kinase subunit β; NFκB, nuclear factor-κB; MCP1, C-C motif chemokine ligand 2; TNFα, tumor necrosis factor α; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; BAMBI, BMP and activin membrane-bound inhibitor; TGFβ, transforming growth factor β1; TGFβRI, TGFβ receptor 1; Smad2, SMAD family member 2; CKI, choline kinase α; LRP5, LDL receptor-related protein 5; GSK-3β, glycogen synthase kinase 3 β; APC, APC regulator of WNT signaling pathway; TCF4, transcription factor 4; CDKs, cyclin-dependent kinases; ECM, extracellular matrix.

#### 4. Role of BAMBI in biological processes and diseases

**Adipogenesis.** Obesity occurs due to an increase in the ratio of caloric intake to energy expenditure, which leads to adipocyte hypertrophy (49). The role of BAMBI in lipid metabolism is relevant to research into obesity and metabolic syndrome, as there is evidence that BAMBI may be involved in regulating adipocyte differentiation and lipid metabolism pathways, affecting energy homeostasis and lipid storage (4,50). One study showed that BAMBI expression was significantly lower in adipose tissue from patients with obesity than in individuals of healthy weight, and 12 specific variant sites in *BAMBI*, including R151W and H201R, were associated with obesity, indicating a strong relationship between BAMBI and obesity (50). In this study, involving 677 children and adolescents with obesity and 529 lean control individuals, researchers conducted mutation analysis of the *BAMBI* coding region and intron-exon boundaries, and identified coding region variants in 21 individuals with obesity compared with 5 lean controls; the difference in variant frequency (3.1% in subjects with obesity and 0.9% in lean controls) was significant ( $P=0.004$ ). However, there have been few studies on the role of BAMBI in adiposity to date, and the role of BAMBI in adipogenesis has been primarily explored at the cellular level. In 2012, Luo *et al* (51) found that BAMBI could act as a proximal effector of fibroblast growth factor 1 (*FGF1*)

in human adipocytes, and was also regulated by *FGF1*, with BAMBI levels decreasing in cells that were treated with *FGF1*. In addition, PI3K and ERK signaling pathways can also regulate BAMBI expression, and TGFβ and Wnt/β-catenin signaling pathways are affected by *BAMBI*, thus inhibiting adipogenesis. In addition, Mai *et al* (52) found that *BAMBI* was downregulated during porcine preadipocyte differentiation. *BAMBI* inhibition increased adipogenesis, primarily through the Wnt/β-catenin signaling pathway. The opposite phenomenon was observed in *BAMBI* overexpressing cells, where a significant increase in nuclear translocation of β-catenin was observed, which inhibited adipogenic differentiation of adipocytes. By contrast, Huang *et al* (53) conducted dual luciferase reporter assays, which demonstrated that BAMBI was a target gene of *miR-106a* during porcine preadipocytes differentiation, and that *miR-106a* promotes lipogenic differentiation of porcine preadipocytes by targeting *BAMBI*. Yang *et al* (54) demonstrated that *BAMBI* gene downregulation promoted bovine preadipocyte differentiation and inhibited the myogenesis of myoblasts. In our latest study (4), adipose-specific *BAMBI* knockout mice (*BAMBI* AKO mice) were constructed. Phenotypically, the *BAMBI* AKO mice exhibited an obese phenotype after high-fat feeding, accompanied by insulin resistance and a fatty liver phenotype. In addition, *BAMBI* knockdown promotes the lipogenic differentiation of white and brown precursor adipocytes. Mechanistically, *BAMBI* deletion

caused an increased in reactive oxygen species (ROS) levels in mitochondria and promoted the mitotic clonal expansion stage of preadipocyte differentiation, which in turn increased binding of CCAAT/Enhancer-Binding Protein  $\beta$  (*C/EBP $\beta$* ) to downstream target genes and ultimately promoted lipogenesis, representing a possible mechanism of adipogenesis regulation through modulation of ROS levels in mice (4).

There is compelling evidence that non-alcoholic fatty liver disease (NAFLD) is more common in individuals who are overweight/obese, and the condition is closely associated with lipid metabolism dysfunction in the liver (55,56). Hepatic steatosis is a relatively benign stage of NAFLD, but can leave the liver vulnerable to further damage (57). Non-alcoholic steatohepatitis (NASH) is characterized by liver inflammation and can progress to liver fibrosis, cirrhosis and hepatocellular carcinoma (58). One study reported that *BAMBI* protein levels were low in human hepatic steatosis and that *BAMBI* levels in the liver negatively correlated with body mass index (BMI) (28). By contrast, in a high-fat diet NASH model, immunohistochemical analysis revealed a significant decrease in *BAMBI* protein levels in the liver (59). In addition, researchers reported a significant increase in *BAMBI* protein levels in experimental NAFLD, and improved hepatic oxidative stress and immune cell function after inhibition of the TLR4 signaling pathway with sparstolonin (59). Furthermore, Chen *et al* (4) showed that *BAMBI* gene deficiency may indirectly cause hepatic steatosis by promoting the release of fatty acids from hypertrophic adipose tissue, rather than by directly regulating adipogenesis in the liver. In conclusion, there is a large body of evidence suggesting that adipose tissue dysfunction is a key factor in NAFLD pathogenesis, and that NAFLD leads to downregulation of *BAMBI*, while *BAMBI* deficiency also causes NAFLD in high-fat diet models.

**Myogenesis.** *BAMBI* is also implicated in myogenesis, muscle tissue maintenance and repair, possibly through effects on muscle stem cell proliferation and differentiation (5,60). Numerous studies have reported that both the TGF $\beta$  and the Wnt/ $\beta$ -catenin pathways have regulatory roles during myogenesis, while *BAMBI* can inhibit signal transduction via these pathways in various cell types. Therefore, investigation of the contribution of *BAMBI* to skeletal muscle myogenesis is warranted. Zhang *et al* (60) showed that *BAMBI* expression levels peaked during the early differentiation stage of C2C12 myoblasts, while interfering with *BAMBI* expression using siRNA inhibited C2C12 myoblast differentiation and Wnt/ $\beta$ -catenin pathway activity. It was concluded that *BAMBI* is required for C2C12 myoblast differentiation and that its role in myogenesis is mediated by the Wnt/ $\beta$ -catenin pathway. In general, *in vivo* studies provide better evidence for the effects of genes on biological processes. Yao *et al* (5) found that *BAMBI* expression levels decreased gradually during skeletal muscle development. Moreover, *BAMBI* was generally expressed at higher levels in glycolytic muscle fibers.

**Ovarian function.** *BAMBI* may also be associated with germ cell development and normal function of ovarian tissue, and its aberrant expression can affect ovary-related reproductive physiological processes (6,61). In the context of reproduction, the results of the study by Bai *et al* (62) elegantly illustrated

the role played by *BAMBI* in pig and human granulosa cells. First, the results revealed that *BAMBI* overexpression promoted the expression of aromatase and steroidogenic acute regulatory protein (*StAR*) in porcine primary granulosa cells, while mRNA and protein levels of P450<sub>scc</sub> and 3 $\beta$ -HSD were not significantly increased. In addition, levels of estradiol and progesterone in the culture medium were significantly increased. Meanwhile, knockdown of endogenous *BAMBI* reduced the mRNA expression levels of cytochrome P450 family 19 subfamily A member 1 (*Cyp19a1*) and *StAR*, as well as the estradiol and progesterone accumulation levels. In human granulosa cells, Bai *et al* (13) reported that *BMP2* activated *SMAD1/5/8* and upregulated *BAMBI* expression, suggesting that *BAMBI* is a BMP target gene in human ovarian granulosa cells that mediates negative feedback regulation of TGF $\beta$  signaling in human ovaries. In another study, Bai *et al* (63) found that stimulation of porcine granulosa cells with *FSH* reduced *BAMBI* expression levels, and concluded that *FSH* can inhibit *BAMBI* expression in porcine luteinized granulosa cells.

Studies in porcine granulosa cells suggested that *BAMBI* is involved in steroid synthesis, which is an important physiological process affecting follicular maturation and ovulation, and granulosa cells are the main cell type in follicles that produce steroid hormones in response to *FSH* and luteinizing hormone stimulation (64,65), while TGF $\beta$ 1 induces *SMAD3* phosphorylation in porcine granulosa cells (62). Pre-transfection with *BAMBI*-overexpression adenovirus inhibited TGF $\beta$ 1-induced downregulation of estradiol and progesterone production, and TGF $\beta$ 1-induced *SMAD3* phosphorylation in porcine granulosa cells (62). In cattle, TGF $\beta$ 1 concentration was negatively correlated with estradiol in follicular fluid, and TGF $\beta$ 1 decreased FSH-stimulated estradiol production in cultured granulosa cells (66). These findings revealed a potential mechanism by which *BAMBI* can regulate steroidogenesis in porcine granulocytes.

Pigs possess genetic and protein variants similar to those of humans, including genes associated with a number of human diseases, such as Alzheimer's disease, Parkinson's disease and obesity (67,68). In addition, pig internal organs are arranged very similarly to those of humans, and pig hearts are comparable in size and shape to those of humans. Although there are similarities between humans and pigs at the genetic level, there are also some differences (69). Overall, however, the similarities are helpful for the study of human disease models, as well as for other aspects of biomedical research, and the pig as a model animal helps to further characterize human disease and physiology (70). To conclude, the role of *BAMBI* in enhancing porcine reproductive performance was reviewed and, given the homology between human and porcine genes and proteins, it is hypothesized that *BAMBI* is a potential physiological target for enhancing female fertility and the treatment of infertility.

**Inflammation.** *BAMBI* has been found to be involved in regulating inflammatory responses. It may play a role in inhibiting or modulating inflammatory processes with potential anti-inflammatory effects. Yang *et al* (7) showed that *BAMBI* expression was significantly decreased in a rat model of spinal cord injury. Overexpression of *BAMBI* effectively reduced the expression of the mammalian target of rapamycin gene,

interleukin 1 $\beta$  (*IL1 $\beta$* ), *IL6* and *IL10*. These results suggested that *BAMBI* has neuroprotective effects in rats with spinal cord injury and can reduce the inflammatory response in rats. If these results are validated in humans, it will be a new therapeutic option to alleviate the neurological damage and inflammatory response caused by spinal cord injury. MCP1 has been recognized as a key mediator of renal fibrosis in chronic kidney diseases, including diabetic nephropathy (71). The reduction of MCP1 expression level implies that the level of inflammation as well as the level of fibrosis in the kidneys is also further reduced (72). In rat renal tubular epithelial cells, interference with *BAMBI* promoted *ERK1/2* phosphorylation and TGF $\beta$ 1-induced monocyte chemotactic protein 1 (*MCP1*) expression. Conversely, *BAMBI* overexpression inhibited *ERK1/2* phosphorylation and TGF $\beta$ 1-induced MCP1 upregulation. Therefore, Liang *et al* (73) suggested that in rat renal tubular epithelial cells, metformin can inhibit TGF $\beta$ 1-induced MCP1 expression through a *BAMBI*-mediated *MEK/ERK1/2* signaling pathway. Studies have also reported on the questionable efficacy of metformin against cisplatin-induced renal cytotoxicity. The results of the study by Li *et al* (74) suggested that metformin may prevent cisplatin-induced tubular cell apoptosis and acute kidney injury by stimulating AMPK $\alpha$  activation and inducing tubular cell autophagy. Given this effect of metformin, the upregulation of *BAMBI* by metformin could also alleviate cisplatin-induced renal cytotoxicity by inhibiting the TGF $\beta$  signaling pathway. In addition, *BAMBI* can regulate the inflammatory responses in different tissues (e.g., glioma and lung) by directly influencing macrophage proliferation and differentiation (75,76). Furthermore, *BAMBI* overexpression reduced the expression of TGF $\beta$ , *IL1 $\beta$* , *IL6* and *IL10* levels, suggesting that *BAMBI* can play a neuroprotective role by reducing inflammatory responses in rats with spinal cord injury (7).

**Tumor development.** Numerous studies have reported that *BAMBI* has an important regulatory role in pathological processes such as tumorigenesis and fibrogenesis, and is highly expressed in various tumor cells and tissues, including ovarian cancer cells and metastatic tumors (45,77). Human *BAMBI* gene expression is downregulated in metastatic melanoma cell lines and high-grade bladder cancer cells (2,78). Furthermore, the inhibitory effect of *BAMBI* promoter hypermethylation on *BAMBI* expression is an important epigenetic event affecting bladder cancer cell invasion (78). In this study, hypermethylation of the *BAMBI* promoter suppressed the expression level of *BAMBI*, whereas a decrease in the expression level of *BAMBI* activated the TGF $\beta$  pathway, which enabled the promotion of tumor cell motility, invasion and survival. In an *in vitro* study of *BAMBI*, injection of colon cancer cells transfected with *BAMBI* overexpression plasmid into the spleen tissue of nude mice resulted in rapid tumor formation and metastasis of colon cancer cells to mouse liver and lymph nodes. Further investigation of the regulatory mechanisms involved revealed that high *BAMBI* expression in colon and liver cancer cells impaired TGF $\beta$ -mediated inhibition of cancer cell growth (46). *BAMBI* has also been reported to promote the growth and invasion of human gastric cancer cells (42,79,80). Yuan *et al* (80) showed that *BAMBI* overexpression inhibited the TGF $\beta$ /epithelial mesenchymal transition signaling pathway and suppressed

the invasiveness of gastric tumors, whereas Zhang *et al* (6) demonstrated that *BAMBI* and *SMAD7* expression levels were both significantly elevated in human gastric cancer tissues. Moreover, *BAMBI* and *SMAD7* inhibited the phosphorylation of *SMAD2*, and decreased phosphorylated *SMAD2* levels were associated with tumor invasion and poor prognosis in patients with gastric cancer (7,11,12). These findings suggested that *BAMBI* and *SMAD7* may synergistically inhibit TGF $\beta$  signaling, thereby promoting gastric cancer progression. In addition, Liu *et al* (42) found that *BAMBI* could also improve the prognosis of gastric cancer by regulating the classical Wnt/ $\beta$ -catenin pathway. To summarize, *BAMBI* can regulate the growth and invasion of gastric cancer cells by modulating TGF $\beta$  and Wnt/ $\beta$ -catenin signaling. By contrast, expression levels of the long non-coding RNA *PVT1* and *BAMBI* were significantly increased during non-small cell lung cancer development, and *PVT1* could promote cell viability, migration and invasion through miR-17-5p targeting of *BAMBI*, thus promoting non-small cell lung cancer development (81).

Besides being involved in cancer development, *BAMBI* is also associated with diseases such as liver fibrosis. A previous study reported that *BAMBI* expression was negatively correlated with donor BMI and was expressed at low levels in human fibrosis-prone fatty liver lesions (28). Furthermore, *BAMBI* was downregulated in rodent models of liver inflammation and fibrosis, and that *BAMBI* and *TLR4* were downregulated in LPS-regulated liver fibrosis; however, hepatoprotective adiponectin induced high *BAMBI* expression in human primary hepatocytes (28). *BAMBI* mRNA and protein levels were significantly reduced in patients with advanced liver fibrosis, while *BAMBI* overexpression decreased the mRNA levels of the fibrosis markers  $\alpha$ -SMA, *COL1* and matrix metalloprotein in human HSCs (26). The aforementioned summarized studies suggest that *BAMBI* may contribute to inflammatory and fibrotic responses in various diseases.

**Embryogenesis.** Several studies have reported important regulatory roles for *BAMBI* in embryonic development and organogenesis (82-84), although a previous *BAMBI* knockout mouse study reported that *BAMBI* is not essential for embryonic development and postnatal survival (84). Onichtchouk *et al* (1) showed that *BAMBI* was closely co-expressed with *BMP4* in the early development of the African toad embryo, while another group found that *BAMBI* expression is regulated by *BMP4* in mouse embryonic fibroblasts, and that *BAMBI* spatiotemporal expression patterns are consistent with those of *BMP4* during mouse embryonic development (84).

In addition to regulating embryonic development, *BAMBI* also regulates tooth formation. Gonzales *et al* (85) reported that *BAMBI* expression is elevated during tooth formation and is involved in regulating the expression of the dental matrix proteins in the MD10-A2 cell line, which normally regulate the control of dentin mineralization (3). Furthermore, Xavier *et al* (86) found that *BAMBI* was expressed in kidney endothelial cells and human umbilical vein endothelial cells, and inhibited endothelial cell function. Furthermore, *BAMBI* knockdown increased capillary generation and migration, while *BAMBI* overexpression



inhibited capillary generation and migration, and an *in vivo* experiment showed that angiogenesis was increased in *BAMBI* knockout mice (14).

In conclusion, dysregulation of *BAMBI* affects the TGF $\beta$  pathway, which may lead to developmental disorders or diseases; however, to date, no specific syndromes or disorders have been associated with *BAMBI* dysregulation during embryonic development. Therefore, further basic and clinical studies are needed to determine whether any syndromes or diseases are associated with *BAMBI* dysregulation.

## 5. Conclusions and perspectives

Recent trends in molecular medicine have centered on the elaboration of the signaling pathways that control the functions of different tissues. Understanding these pathways can facilitate treatment, as well as prevention, of a wide range of pathologies arising from abnormal molecular mechanisms. Since its discovery in 1998, *BAMBI* has attracted considerable attention, due to its involvement in various pathophysiological processes associated with a number of diseases. In the present review, the major physiological signaling pathways involving the function of *BAMBI* and its associations with a range of diseases were evaluated. As the role of *BAMBI* in regulating lipid metabolism remains controversial, due to its inhibitory effect on TGF $\beta$ , more research is needed to determine whether *BAMBI* influences the development of obesity and diabetes through TGF $\beta$  signaling. Although previous studies have reported a key role for *BAMBI* in lipid metabolism, some conflicting results require further discussion and clarification. Firstly, little is known about the mechanisms that regulate *BAMBI* in various diseases. Identification of key regulators may provide new insights into the physiological functions of *BAMBI* and has potential to facilitate development of *BAMBI*-based therapeutic approaches. Secondly, the maximum dose of exogenous *BAMBI* recombinant protein tolerated by patients has not been studied in depth, nor has it been determined whether there are uncontrollable side effects associated with *BAMBI* administration.

In brief, although *BAMBI* can inhibit the occurrence of some tumors and provide protection for human health, at present, *BAMBI* is not in use as part of a specific drug treatment in clinical medicine, and more research is needed to explore this possibility. Although large, complex and accurate gene regulatory networks involving *BAMBI* have been discovered, further investigation is required to provide an evidence base for the application of *BAMBI* as a drug to treat various diseases.

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## Availability of data and materials

Not applicable.

## Authors' contributions

XCC, DZ and QY conceived the manuscript and summarized the contents of the manuscript. XCC, HG and LSZ were responsible for the literature search and discussion. XCC and AQX drafted the manuscript. XCC, JL and PHS wrote the revised manuscript and prepared the figures. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

## Ethics approval and consent to participate

Not applicable.

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Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Onichtchouk D, Chen YG, Dosch R, Gawantka V, Delius H, Massagué J and Niehrs C: Silencing of TGF-beta signalling by the pseudoreceptor BAMBI. *Nature* 401: 480-485, 1999.
2. Degen WG, Wettersman MA, van Groningen JJ, Cornelissen IM, Lemmers JP, Agterbos MA, Geurts van Kessel A, Swart GW and Bloemers HP: Expression of nma, a novel gene, inversely correlates with the metastatic potential of human melanoma cell lines and xenografts. *Int J Cancer* 65: 460-465, 1996.
3. Knight C, Simmons D, Gu TT, Gluhak-Heinrich J, Pavlin D, Zeichner-David M and MacDougall M: Cloning, characterization, and tissue expression pattern of mouse Nma/BAMBI during odontogenesis. *J Dent Res* 80: 1895-1902, 2001.
4. Chen X, Zhao C, Xu Y, Huang K, Wang Y, Wang X, Zhou X, Pang W, Yang G and Yu T: Adipose-specific BMP and activin membrane-bound inhibitor (BAMBI) deletion promotes adipogenesis by accelerating ROS production. *J Biol Chem* 296: 100037, 2021.
5. Yao X, Yu T, Xi F, Xu Y, Ma L, Pan X, Chen S, Han M, Yin Y, Dai X, *et al*: BAMBI shuttling between cytosol and membrane is required for skeletal muscle development and regeneration. *Biochem Biophys Res Commun* 509: 125-132, 2019.
6. Zhang Y, Guo Z, Du Z, Yao Z, Guo T, Cheng Y, Wang K, Ma X, Chen C, Kebreab E, *et al*: Effects of BAMBI on luteinized follicular granulosa cell proliferation and steroid hormone production in sheep. *Mol Reprod Dev* 90: 153-165, 2023.
7. Yang Y, Guo C, Liao B, Cao J, Liang C and He X: BAMBI inhibits inflammation through the activation of autophagy in experimental spinal cord injury. *Int J Mol Med* 39: 423-429, 2017.
8. Weber F, Treeck O, Mester P and Buechler C: Expression and function of BMP and activin membrane-bound inhibitor (BAMBI) in chronic liver diseases and hepatocellular carcinoma. *Int J Mol Sci* 24: 3473, 2023.
9. Pawlak JB and Globe GC: TGF- $\beta$  superfamily co-receptors in cancer. *Dev Dyn* 251: 137-163, 2022.
10. Nickel J, Ten Dijke P and Mueller TD: TGF- $\beta$  family co-receptor function and signaling. *Acta Bioch Bioph Sin (Shanghai)* 50: 12-36, 2018.

11. Kirsch T, Sebald W and Dreyer MK: Crystal structure of the BMP-2-BRIA ectodomain complex. *Nat Struct Biol* 7: 492-496, 2000.
12. Luo W and Lin SC: Axin: A master scaffold for multiple signaling pathways. *Neurosignals* 13: 99-113, 2004.
13. Bai L, Chang HM, Cheng JC, Klausen C, Chu G, Leung PCK and Yang G: SMAD1/5 mediates bone morphogenetic protein 2-induced up-regulation of BAMBI expression in human granulosa-lutein cells. *Cell Signal* 37: 52-61, 2017.
14. Guillot N, Kollins D, Gilbert V, Xavier S, Chen J, Gentle M, Reddy A, Bottinger E, Jiang R, Rastaldi MP, *et al*: BAMBI regulates angiogenesis and endothelial homeostasis through modulation of alternative TGF $\beta$  signaling. *PLoS One* 7: e39406, 2012.
15. Yan X, Lin Z, Chen F, Zhao X, Chen H, Ning Y and Chen YG: Human BAMBI cooperates with Smad7 to inhibit transforming growth factor-beta signaling. *J Biol Chem* 284: 30097-30104, 2009.
16. Paulsen M, Legewie S, Eils R, Karaulanov E and Niehrs C: Negative feedback in the bone morphogenetic protein 4 (BMP4) synexpression group governs its dynamic signaling range and canalizes development. *Proc Natl Acad Sci USA* 108: 10202-10207, 2011.
17. Wu Y, Li Q, Zhou X, Yu J, Mu Y, Munker S, Xu C, Shen Z, Müllenbach R, Liu Y, *et al*: Decreased levels of active SMAD2 correlate with poor prognosis in gastric cancer. *PLoS One* 7: e35684, 2012.
18. Shehata MM, Sallam AM, Naguib MG and El-Mesallamy HO: Overexpression of BAMBI and SMAD7 impacts prognosis of acute myeloid leukemia patients: A potential TERT non-canonical role. *Cancer Biomark* 31: 47-58, 2021.
19. Sekiya T, Oda T, Matsuura K and Akiyama T: Transcriptional regulation of the TGF-beta pseudoreceptor BAMBI by TGF-beta signaling. *Biochem Biophys Res Commun* 320: 680-684, 2004.
20. Kashani B, Zandi Z, Pourbagheri-Sigaroodi A, Bashash D and Ghaffari SH: The role of toll-like receptor 4 (TLR4) in cancer progression: A possible therapeutic target? *J Cell Physiol* 236: 4121-4137, 2021.
21. Medzhitov R, Preston-Hurlburt P and Janeway CA Jr: A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 388: 394-397, 1997.
22. Federico S, Pozzetti L, Papa A, Carullo G, Gemma S, Butini S, Campiani G and Relitti N: Modulation of the innate immune response by targeting toll-like receptors: A perspective on their agonists and antagonists. *J Med Chem* 63: 13466-13513, 2020.
23. Koushki K, Shahbaz SK, Mashayekhi K, Sadeghi M, Zayeri ZD, Taba MY, Banach M, Al-Rasadi K, Johnston TP and Sahebkar A: Anti-inflammatory action of statins in cardiovascular disease: The role of inflammasome and toll-like receptor pathways. *Clin Rev Allergy Immunol* 60: 175-199, 2021.
24. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA and Schwabe RF: TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 13: 1324-1332, 2007.
25. Liu C, Chen X, Yang L, Kisseleva T, Brenner DA and Seki E: Transcriptional repression of the transforming growth factor  $\beta$  (TGF- $\beta$ ) Pseudoreceptor BMP and activin membrane-bound inhibitor (BAMBI) by Nuclear Factor  $\kappa$ B (NF- $\kappa$ B) p50 enhances TGF- $\beta$  signaling in hepatic stellate cells. *J Biol Chem* 289: 7082-7091, 2014.
26. Tao L, Xue D, Shen D, Ma W, Zhang J, Wang X, Zhang W, Wu L, Pan K, Yang Y, *et al*: MicroRNA-942 mediates hepatic stellate cell activation by regulating BAMBI expression in human liver fibrosis. *Arch Toxicol* 92: 2935-2946, 2018.
27. He Y, Ou Z, Chen X, Zu X, Liu L, Li Y, Cao Z, Chen M, Chen Z, Chen H, *et al*: LPS/TLR4 signaling enhances TGF- $\beta$  response through downregulating BAMBI during prostatic hyperplasia. *Sci Rep* 6: 27051, 2016.
28. Wanninger J, Neumeier M, Bauer S, Weiss TS, Eisinger K, Walter R, Dorn C, Hellerbrand C, Schäffler A and Buechler C: Adiponectin induces the transforming growth factor decoy receptor BAMBI in human hepatocytes. *FEBS Lett* 585: 1338-1344, 2011.
29. Dediulia T: Expression analysis and functional studies of Bone Morphogenetic Protein and Activin Membrane-Bound Inhibitor (BAMBI) in hepatocellular carcinoma (unpublished PhD thesis). Ruperto Carola University Heidelberg, Heidelberg, 2019.
30. Tomita K, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, Okada Y, Kurihara C, Irie R, Yokoyama H, *et al*: Free cholesterol accumulation in hepatic stellate cells: Mechanism of liver fibrosis aggravation in nonalcoholic steatohepatitis in mice. *Hepatology* 59: 154-169, 2014.
31. Tomita K, Teratani T, Suzuki T, Shimizu M, Sato H, Narimatsu K, Usui S, Furuhashi H, Kimura A, Nishiyama K, *et al*: Acyl-CoA: Cholesterol acyltransferase 1 mediates liver fibrosis by regulating free cholesterol accumulation in hepatic stellate cells. *J Hepatol* 61: 98-106, 2014.
32. Chen M, Liu J, Yang W and Ling W: Lipopolysaccharide mediates hepatic stellate cell activation by regulating autophagy and retinoic acid signaling. *Autophagy* 13: 1813-1827, 2017.
33. MacDonald BT, Tamai K and He X: Wnt/beta-catenin signaling: Components, mechanisms, and diseases. *Dev Cell* 17: 9-26, 2009.
34. Perugorria MJ, Olaizola P, Labiano I, Esparza-Baquer A, Marziani M, Marin JJG, Bujanda L and Banales JM: Wnt-beta-catenin signalling in liver development, health and disease. *Nat Rev Gastroenterol Hepatol* 16: 121-136, 2019.
35. Cong F, Schweizer L and Varmus H: Wnt signals across the plasma membrane to activate the beta-catenin pathway by forming oligomers containing its receptors, Frizzled and LRP. *Development* 131: 5103-5115, 2004.
36. Zeng X, Tamai K, Doble B, Li S, Huang H, Habas R, Okamura H, Woodgett J and He X: A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature* 438: 873-877, 2005.
37. Davidson G, Wu W, Shen J, Bilic J, Fenger U, Stanek P, Glinka A and Niehrs C: Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction. *Nature* 438: 867-872, 2005.
38. Schwarz-Romond T, Fiedler M, Shibata N, Butler PJ, Kikuchi A, Higuchi Y and Bienz M: The DIX domain of Dishevelled confers Wnt signaling by dynamic polymerization. *Nat Struct Mol Biol* 14: 484-492, 2007.
39. Gao C and Chen YG: Dishevelled: The hub of Wnt signaling. *Cell Signal* 22: 717-727, 2010.
40. Vlad A, Rohrs S, Klein-Hitpass L and Muller O: The first five years of the Wnt targetome. *Cell Signal* 20: 795-802, 2008.
41. Lu HJ, Yan J, Jin PY, Zheng GH, Zhang HL, Bai M, Wu DM, Lu J and Zheng YL: Mechanism of MicroRNA-708 Targeting BAMBI in cell proliferation, migration, and apoptosis in mice with melanoma via the Wnt and TGF- $\beta$  signaling pathways. *Technol Cancer Res Treat* 17: 1533034618756784, 2018.
42. Liu K, Song X, Ma H, Liu L, Wen X, Yu J, Wang L and Hu S: Knockdown of BAMBI inhibits  $\beta$ -catenin and transforming growth factor  $\beta$  to suppress metastasis of gastric cancer cells. *Mol Med Rep* 10: 874-880, 2014.
43. Yuan H, Liu H, Liu Z, Zhu D, Amos CI, Fang S, Lee JE and Wei Q: Genetic variants in Hippo pathway genes YAP 1, TEAD 1 and TEAD 4 are associated with melanoma-specific survival. *Int J Cancer* 137: 638-645, 2015.
44. Zhao HJ, Chang HM, Klausen C, Zhu H, Li Y and Leung PCK: Bone morphogenetic protein 2 induces the activation of WNT/ $\beta$ -catenin signaling and human trophoblast invasion through up-regulating BAMBI. *Cell Signal* 67: 109489, 2020.
45. Fritzmann J, Morkel M, Besser D, Budczies J, Kosel F, Brembeck FH, Stein U, Fichtner I, Schlag PM and Birchmeier W: A colorectal cancer expression profile that includes transforming growth factor beta inhibitor BAMBI predicts metastatic potential. *Gastroenterology* 137: 165-175, 2009.
46. Sekiya T, Adachi S, Kohu K, Yamada T, Higuchi O, Furukawa Y, Nakamura Y, Nakamura T, Tashiro K, Kuhara S, *et al*: Identification of BMP and activin membrane-bound inhibitor (BAMBI), an inhibitor of transforming growth factor-beta signaling, as a target of the beta-catenin pathway in colorectal tumor cells. *J Biol Chem* 279: 6840-6846, 2004.
47. Subramaniam N, Sherman MH, Rao R, Wilson C, Coulter S, Atkins AR, Evans RM, Liddle C and Downes M: Metformin-mediated Bambi expression in hepatic stellate cells induces pro-survival Wnt/beta-catenin signaling. *Cancer Prev Res (Phila)* 5: 553-561, 2012.
48. Zhou L, Park J, Jang KY, Park HS, Wagle S, Yang KH, Lee KB, Park BH and Kim JR: The overexpression of BAMBI and its involvement in the growth and invasion of human osteosarcoma cells. *Oncol Rep* 30: 1315-1322, 2013.
49. Piché ME, Tchernof A and Després JP: Obesity phenotypes, diabetes, and cardiovascular diseases. *Circ Res* 126: 1477-1500, 2020.
50. Van Camp JK, De Freitas F, Zegers D, Beckers S, Verhulst SL, Van Hoorenbeeck K, Massa G, Verrijken A, Desager KN, Van Gaal LF and Van Hul W: Investigation of common and rare genetic variation in the BAMBI genomic region in light of human obesity. *Endocrine* 52: 277-286, 2016.



51. Luo X, Hutley LJ, Webster JA, Kim YH, Liu DF, Newell FS, Widberg CH, Bachmann A, Turner N, Schmitz-Peiffer C, *et al*: Identification of BMP and activin membrane-bound inhibitor (BAMBI) as a potent negative regulator of adipogenesis and modulator of autocrine/paracrine adipogenic factors. *Diabetes* 61: 124-136, 2012.
52. Mai Y, Zhang Z, Yang H, Dong P, Chu G, Yang G and Sun S: BMP and activin membrane-bound inhibitor (BAMBI) inhibits the adipogenesis of porcine preadipocytes through Wnt/ $\beta$ -catenin signaling pathway. *Biochem Cell Biol* 92: 172-182, 2014.
53. Huang K, Shi X, Wang J, Yao Y, Peng Y, Chen X, Li X and Yang G: Upregulated microRNA-106a promotes porcine preadipocyte proliferation and differentiation by targeting different genes. *Genes (Basel)* 10: 805, 2019.
54. Yang X, Ning Y, Mei C, Zhang W, Sun J, Wang S and Zan L: The role of BAMBI in regulating adipogenesis and myogenesis and the association between its polymorphisms and growth traits in cattle. *Mol Biol Rep* 47: 5963-5974, 2020.
55. Polyzos SA, Kountouras J and Mantzoros CS: Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. *Metabolism* 92: 82-97, 2019.
56. Ferguson D and Finck BN: Emerging therapeutic approaches for the treatment of NAFLD and type 2 diabetes mellitus. *Nat Rev Endocrinol* 17: 484-495, 2021.
57. Pouwels S, Sakran N, Graham Y, Leal A, Pinter T, Yang W, Kassir R, Singhal R, Mahawar K and Ramnarain D: Non-alcoholic fatty liver disease (NAFLD): A review of pathophysiology, clinical management and effects of weight loss. *BMC Endocr Disord* 22: 63, 2022.
58. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J and Bugianesi E: Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 15: 11-20, 2018.
59. Dattaroy D, Seth RK, Sarkar S, Kimono D, Albadrani M, Chandrashekar V, Hasson FA, Singh UP, Fan D, Nagarkatti M, *et al*: Sparstolonin B (SsnB) attenuates liver fibrosis via a parallel conjugate pathway involving P53-P21 axis, TGF-beta signaling and focal adhesion that is TLR4 dependent. *Eur J Pharmacol* 841: 33-48, 2018.
60. Zhang Q, Shi XE, Song C, Sun S, Yang G and Li X: BAMBI Promotes C2C12 myogenic differentiation by enhancing Wnt/ $\beta$ -Catenin Signaling. *Int J Mol Sci* 16: 17734-17745, 2015.
61. Loveland KL, Bakker M, Meehan T, Christy E, von Schönfeldt V, Drummond A and de Kretser D: Expression of Bambi is widespread in juvenile and adult rat tissues and is regulated in male germ cells. *Endocrinology* 144: 4180-4186, 2003.
62. Bai L, Chu G, Wang W, Xiang A and Yang G: BAMBI promotes porcine granulosa cell steroidogenesis involving TGF- $\beta$  signaling. *Theriogenology* 100: 24-31, 2017.
63. Bai L, Chu G, Mai Y, Zheng J, Wang W, Zhang Q and Yang G: Identification and expression analyses of BAMBI mediated by FSH in swine luteinizing granulosa cells. *Theriogenology* 82: 1094-1101, 2014.
64. Baufeld A and Vanselow J: Increasing cell plating density mimics an early post-LH stage in cultured bovine granulosa cells. *Cell Tissue Res* 354: 869-880, 2013.
65. Matsuda F, Inoue N, Manabe N and Ohkura S: Follicular growth and atresia in mammalian ovaries: Regulation by survival and death of granulosa cells. *J Reprod Dev* 58: 44-50, 2012.
66. Ouellette Y, Price CA and Carriere PD: Follicular fluid concentration of transforming growth factor-beta1 is negatively correlated with estradiol and follicle size at the early stage of development of the first-wave cohort of bovine ovarian follicles. *Domest Anim Endocrinol* 29: 623-633, 2005.
67. Prather RS, Lorson M, Ross JW, Whyte JJ and Walters E: Genetically engineered pig models for human diseases. *Annu Rev Anim Biosci* 1: 203-219, 2013.
68. Perleberg C, Kind A and Schnieke A: Genetically engineered pigs as models for human disease. *Dis Model Mech* 11: dmm030783, 2018.
69. Lunney JK, Van Goor A, Walker KE, Hailstock T, Franklin J and Dai C: Importance of the pig as a human biomedical model. *Sci Transl Med* 13: eabd5758, 2021.
70. Hou N, Du X and Wu S: Advances in pig models of human diseases. *Animal Model Exp Med* 5: 141-152, 2022.
71. Du Q, Fu YX, Shu AM, Lv X, Chen YP, Gao YY, Chen J, Wang W, Lv GH, Lu JF and Xu HQ: Loganin alleviates macrophage infiltration and activation by inhibiting the MCP-1/CCR2 axis in diabetic nephropathy. *Life Sci* 272: 118808, 2021.
72. Tesch GH: MCP-1/CCL2: A new diagnostic marker and therapeutic target for progressive renal injury in diabetic nephropathy. *Am J Physiol Renal Physiol* 294: F697-F701, 2008.
73. Liang D, Song Z, Liang W, Li Y and Liu S: Metformin inhibits TGF-beta 1-induced MCP-1 expression through BAMBI-mediated suppression of MEK/ERK1/2 signalling. *Nephrology (Carlton)* 24: 481-488, 2019.
74. Li J, Gui Y, Ren J, Liu X, Feng Y, Zeng Z, He W, Yang J and Dai C: Metformin protects against cisplatin-induced tubular cell apoptosis and acute kidney injury via AMPK $\alpha$ -regulated autophagy induction. *Sci Rep* 6: 23975, 2016.
75. Wang D, Chen X and Zhang R: BAMBI promotes macrophage proliferation and differentiation in gliomas. *Mol Med Rep* 17: 3960-3966, 2018.
76. Sun SW, Chen L, Zhou M, Wu JH, Meng ZJ, Han HL, Miao SY, Zhu CC and Xiong XZ: BAMBI regulates macrophages inducing the differentiation of Treg through the TGF- $\beta$  pathway in chronic obstructive pulmonary disease. *Respir Res* 20: 26, 2019.
77. Pils D, Wittlinger M, Petz M, Gugerell A, Gregor W, Alfaniz A, Horvat R, Braicu EI, Sehoul J, Zeillinger R, *et al*: BAMBI is overexpressed in ovarian cancer and co-translocates with Smads into the nucleus upon TGF-beta treatment. *Gynecol Oncol* 117: 189-197, 2010.
78. Khin SS, Kitazawa R, Win N, Aye TT, Mori K, Kondo T and Kitazawa S: BAMBI gene is epigenetically silenced in subset of high-grade bladder cancer. *Int J Cancer* 125: 328-338, 2009.
79. Sasaki T, Sasahira T, Shimura H, Ikeda S and Kuniyasu H: Effect of Nma on growth inhibition by TGF-beta in human gastric carcinoma cell lines. *Oncol Rep* 11: 1219-1223, 2004.
80. Yuan CL, Liang R, Liu ZH, Li YQ, Luo XL, Ye JZ and Lin Y: Bone morphogenetic protein and activin membrane-bound inhibitor overexpression inhibits gastric tumor cell invasion via the transforming growth factor- $\beta$ /epithelial-mesenchymal transition signaling pathway. *Exp Ther Med* 15: 5422-5430, 2018.
81. Wang Z, Zhang Q, Sun Y and Shao F: Long Non-Coding RNA PVT1 Regulates BAMBI to promote tumor progression in non-small cell lung cancer by sponging miR-17-5p. *Onco Targets Ther* 13: 131-142, 2020.
82. Grotewold L, Plum M, Dildrop R, Peters T and Rüther U: Bambi is coexpressed with Bmp-4 during mouse embryogenesis. *Mech Dev* 100: 327-330, 2001.
83. Chen J, Bush JO, Ovitt CE, Lan Y and Jiang R: The TGF-beta pseudoreceptor gene Bambi is dispensable for mouse embryonic development and postnatal survival. *Genesis* 45: 482-486, 2007.
84. Higashihori N, Song Y and Richman JM: Expression and regulation of the decoy bone morphogenetic protein receptor BAMBI in the developing avian face. *Dev Dyn* 237: 1500-1508, 2008.
85. Gonzales CB, Simmons D and MacDougall M: Competing roles of TGFbeta and Nma/BAMBI in odontoblasts. *J Dent Res* 89: 597-602, 2010.
86. Xavier S, Gilbert V, Rastaldi MP, Krick S, Kollins D, Reddy A, Bottinger E, Cohen CD and Schlondorff D: BAMBI is expressed in endothelial cells and is regulated by lysosomal/autolysosomal degradation. *PLoS One* 5: e12995, 2010.

