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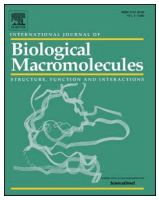

Activin B participates in ovarian development in Japanese eel (*Anguilla japonica*) via coordinated regulation of reproductive signaling and metabolic pathways.

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ABSTRACT

The Japanese eel (*Anguilla japonica*) is a commercially valuable catadromous species widely cultured in East Asia. However, large-scale artificial propagation remains challenging. Activin B, a dimeric protein belonging to the transforming growth factor-β (TGF-β) superfamily, plays key roles in vertebrate gonadal development, yet its function in eel ovarian development has not been characterized. In this study, we cloned the *inhbb* gene from Japanese eel, expressed its recombinant Activin B protein, and evaluated its effects on ovarian development under artificial induction conditions. Adult females induced into vitellogenesis with human chorionic gonadotropin (hCG) were administered recombinant Activin B or saline for eight weeks. Integrated analyses combining serum hormone measurements, transcriptomics, and untargeted metabolomics revealed that Activin B significantly elevated estradiol levels and regulated multiple reproductive signaling pathways—including Smad, MAPK, GnRH, insulin, VEGF, and FoxO—as well as key metabolic processes related to steroid, lipid, and amino acid metabolism. These findings highlight the endocrine–metabolic coordination of Activin B in ovarian development, which could offer a theoretical basis for artificial reproduction in Japanese eel.

1. Introduction

In teleosts, gonadotropins are the primary endocrine regulators of gonadal development, promoting the proliferation and differentiation of both somatic and germ cells. In addition to these systemic hormones, a range of intra-gonadal factors—often members of the transforming growth factor-β (TGF-β) superfamily—play critical roles in the local regulation of folliculogenesis, steroidogenesis, and gametogenesis [1]. These locally secreted modulators act in a paracrine or autocrine manner, orchestrating stage-specific events within the gonads and ensuring the precise progression of reproductive development [2,3]. The interplay between endocrine signals and local regulators is therefore essential for maintaining reproductive competence [4].

Among these local factors, Activin B, a homodimer composed of two βB subunits encoded by the *inhbb* gene, has emerged as a key regulator of gonadal function in vertebrates [5,6]. In mammals, Activin B is predominantly expressed in granulosa cells of the ovary and Sertoli cells of the testis, where it enhances the expression of follicle-stimulating hormone receptor (FSHR), stimulates aromatase activity, and promotes steroidogenesis and germ cell development via the Smad2/3 signaling pathway [7–9]. In addition, it also participates in the fine-tuning of the hypothalamic–pituitary–gonadal (HPG) axis by providing positive feedback on follicle-stimulating hormone (FSH) synthesis [10,11]. In teleosts, *inhbb* exhibits dynamic expression patterns within the gonads, closely associated with specific stages of oogenesis and spermatogenesis. [12,13]. In zebrafish (*Danio rerio*), *inhbb* is detected in both granulosa and Sertoli cells, suggesting a conserved role in gametogenesis [14]. Moreover, studies in goldfish (*Carassius auratus*) further support its involvement in gonadotropin regulation during reproductive cycles [15].

The Japanese eel (*Anguilla japonica*) is a catadromous teleost species that matures in freshwater but undertakes oceanic migration to spawn. It is of high economic significance in East Asian aquaculture, especially in Japan, China, and Korea [16]. However, its complex life cycle presents major challenges for complete artificial reproduction. Although hormonal treatment can successfully induce spawning and fertilization,

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it remains far from sufficient to meet the requirements of large-scale aquaculture. Therefore, investigating intra-ovarian regulatory factors and their roles in the ovarian development of Japanese eel is of great importance for achieving fully artificial large-scale propagation and ultimately meeting the needs of both industry and market.

Given the established roles of Activin B in regulating gonadal development and steroidogenesis, its potential function in ovarian development of the Japanese eel warrants investigation. In this study, we cloned the *inhbb* gene, expressed recombinant Activin B, and evaluated its effects on ovarian development through integrated transcriptomic and metabolomic analyses. Our aim was to elucidate its biological functions and potential regulatory mechanisms, thereby providing meaningful theoretical support for artificial reproduction of the Japanese eel.

2. Materials and methods

2.1. Animals

All the adult female Japanese eels were sourced from an aquaculture farm in Fujian, China. All animal experiments and sample collection procedures were performed in accordance with the respective Animal Research and Ethics Committees of Ocean University of China (Permit Number: 20141201).

2.2. Gene clone and molecular characterization

According to the genome, the open reading frame (ORF) of *inhbb* in Japanese eel, was predicted, and gene-specific primers were designed by primer premier 5 software (Premier, Canada). To amplify the *inhbb* gene sequence of Japanese eel, PCR amplification (Sparkjade, China), product purification (Vazyme, China), and cloning (Vazyme, China) were performed using commercial kits. All procedures were carried out according to the manufacturer's protocols. All primers used in this study are listed in Table S1.

The signal peptide of Activin B was predicted using the SignalP 6.0 Server (http://www.cbs.dtu.dk/services/SignalP-6.0/). Multiple sequence alignment was performed using Clustal X, and phylogenetic analysis of amino acid sequences was conducted with MEGA 7 employing the neighbor-joining method [17,18]. The molecular weight and isoelectric point of Activin B were estimated using the ExPASy Compute pI/MW tool (http://web.expasy.org/compute_pi/).

2.3. Recombinant Activin B prokaryotic expression of Japanese eel

Recombinant Japanese eel Activin B was expressed using the *Escherichia coli* Rosetta-gami B (DE3)/pET expression system (Novagen, Germany). Overlapping primers specific to the *inhbb* of Japanese eel coding region were designed for PCR amplification (Table S1). The PCR product was digested with BamHI and *Kpn*I, and subsequently cloned into the N-His-SUMO-pET expression vector. The resulting plasmid was transformed into *E. coli* Rosetta-gami B (DE3) competent cells. Expression of the recombinant protein and subsequent refolding of inclusion bodies were performed as previously described [19]. The 3D structure of Activin B in Japanese eel was modeled using SWISS-Model (https://swissmodel.expasy.org/).

2.4. Injection and sample collection

After being temporarily reared in seawater for two weeks, ovarian development of Japanese eel was induced by intramuscular injection of human chorionic gonadotropin (hCG; Ningbo Second Hormone Factory, China) at a dose of 300 IU/kg body weight. On the same time, the treatment group ($n = 6$) received weekly intramuscularly injections of recombinant Japanese eel Activin B, while the control group ($n = 6$) was injected with an equal volume of physiological saline. Injections were administered once per week for a total of eight weeks, and the water temperature was maintained at 18–20 °C throughout the experiment. At the end of the experiment, serum samples were collected to measure estradiol (E2) levels by using enzyme-linked immunosorbent assay (ELISA) kits (Runyu, China). Ovarian tissues were sampled for transcriptomic and metabolomic analyses, while fixed ovarian tissues were sectioned and stained with hematoxylin and eosin (H&E) for histological observation and imaging with an Olympus bright field light microscope (Olympus, Japan).

2.5. RNA extraction and transcriptome sequencing

Total RNA from ovarian tissues of both groups was extracted using SparkZol Reagent (Sparkjade, China). A total of eight RNA samples ($n = 4$ per group) were selected for transcriptome sequencing, including four from the recombinant Activin B-treated group (ActB_tis1 to ActB_tis4) and four from the control group (Ctrl_tis1 to Ctrl_tis4). To ensure experimental rigor and data accuracy, all experimental samples were randomly selected. RNA-seq libraries were prepared and sequenced on the Illumina platform, generating 150 bp paired-end reads (NCBI Accession: PRJNA1305659). After filtering out low-quality reads and removing adapters, clean data were aligned to the *Anguilla japonica* reference genome (PRJNA852364) using HISAT2. Transcript assembly and expression quantification were carried out with StringTie. Differential expression analysis was conducted using DESeq2, with significance thresholds set at an absolute $|\log_2\text{FoldChange}| > 1$ and an adjusted pvalue < 0.05. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were subsequently performed to explore the functional categories and signaling pathways associated with the differentially expressed genes (DEGs).

2.6. Metabolite extraction and untargeted LC-MS analysis

A total of twelve ovarian tissue samples ($n = 6$ per group) from the Activin B-treated (ActB_meta_T1–T6) and control (ActB_meta_C1–C6) groups were subjected to untargeted metabolomic profiling using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Raw spectral data were processed with Compound Discoverer, which included peak detection, alignment, retention time calibration, and data normalization. Metabolite annotation was achieved by matching spectral features against both public (e.g., KEGG, HMDB, LIPID MAPS) and in-house databases. Differential metabolites (DMs) were identified using the following thresholds: variable importance in projection (VIP) > 1.0, fold change (FC) > 1.2 or < 0.833, and pvalue < 0.05. To assess metabolic variation between groups, multivariate analyses such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed. KEGG pathway enrichment analysis was subsequently conducted to reveal the biological relevance of the identified metabolites.

2.7. Transcriptome–metabolome integration

To investigate the interplay between gene expression and metabolite alterations during ovarian development, an integrated analysis combining transcriptomic and metabolomic datasets was conducted. Pearson correlation coefficients between DEGs and DMs were calculated using the stats package in R (v4.0.3). Gene–metabolite pairs with a pvalue < 0.05 were considered significantly correlated. To enhance interpretability, the top 50 DEGs and top 50 DMs (ranked by ascending pvalue) were selected for visualization. A correlation heatmap was generated to display the interaction patterns between these selected genes and metabolites. All DEGs and DMs were jointly analyzed for pathway enrichment using the KEGG database via the MetaboAnalyst platform (https://www.metaboanalyst.ca/). Shared KEGG pathways between the transcriptomic and metabolomic datasets were identified by overlapping the pathway enrichment results from both analyses,

which enabled the identification of key biochemical and signaling pathways jointly regulated by DEGs and DMs.

2.8. Statistical analysis

All data were expressed as the mean values ± SEM. Data analyses were performed by *t*-test, and significance was considered at $P < 0.05$. All statistics were tested using SPSS 21.0 statistical software (SPSS, USA).

3. Results

3.1. Molecular characterization and evolutionary analysis of inhbb in Japanese eel

The cloning results revealed that the full-length cDNA sequence of *inhbb* (GenBank accession number: PX060996) was 1182 base pairs (bp), which encoded a protein of 393 amino acids (aa). A signal peptide comprising the first 20 aa was predicted at the N-terminus, as shown in Fig. 1A, B.

The phylogenetic tree showed that Activin B from teleosts and other vertebrates were grouped into two distinct clades. Within the teleost lineage, *Anguilla japonica* shared the closest evolutionary relationship with *Anguilla anguilla*, followed by *Oncorhynchus mykiss*, *Larimichthys crocea*, and *Oreochromis niloticus* (Fig. 1C). As illustrated in Fig. 1D, sequence alignment revealed the presence of a highly conserved mature peptide region in the Activin B across these species, indicating strong evolutionary conservation of this functional domain.

3.2. Construction and expression of recombinant Activin B of Japanese eel

The mature peptide region of Japanese eel Activin B was inserted into the N-His-SUMO-pET expression vector to construct a recombinant protein expression plasmid (Fig. S1). Prokaryotic expression followed by inclusion body refolding yielded a recombinant protein with an approximate molecular weight of 26.4 kDa (Fig. 2). The predicted three-dimensional structure of the protein is shown in Fig. S1.

3.3. Activin B enhances E2 production in Japanese eel

Ovarian tissues from all experimental fish were subjected to H&E staining, and two samples from each group were randomly selected for presentation. Histological observations showed that the ovaries in both

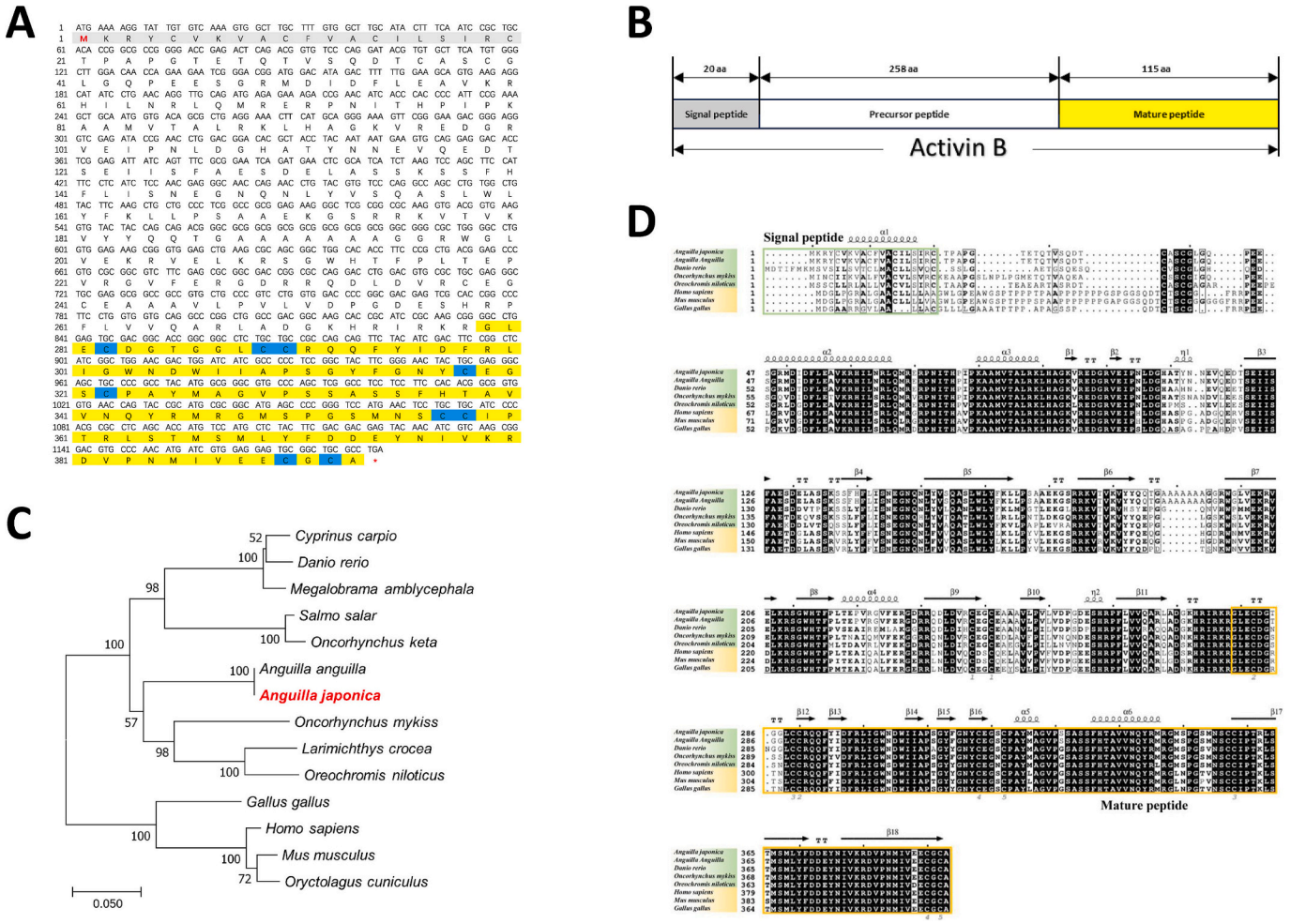

Fig. 1. (A) Nucleotide and amino acid sequences of *inhbb* in Japanese eel (Gray: Signal peptide; Yellow: Mature peptide; Blue: Cysteine.). (B) Structural composition of Activin B in Japanese eel. (C) A phylogenetic tree was constructed using the neighbor-joining method in Japanese eel and other species. Data were resampled with 1000 bootstrap replicates. Accession numbers: *Cyprinus carpio* (AOW71516.1), *Danio rerio* (NP_571143.2), *Megalobrama amblycephala* (XP_048049468.1), *Salmo salar* (XP_014029178.1), *Oncorhynchus keta* (XP_035605606.1), *Anguilla anguilla* (XP_035264053.1), *Oncorhynchus mykiss* (XP_021427073.2), *Larimichthys crocea* (XP_010744581.1), *Oreochromis niloticus* (XP_019207789.1), *Gallus gallus* (NP_990537.2), *Homo sapiens* (KAI4036073.1), *Mus musculus* (NP_032407.1), *Oryctolagus cuniculus* (XP_008256688.1). (D) The sequence alignment of Activin B in Japanese eel (*Anguilla japonica*), European eel (*Anguilla anguilla*), zebrafish (*Danio rerio*), rainbow trout (*oncorhynchus mykiss*), Nile tilapia (*Oreochromis niloticus*), human (*Homo sapiens*), mouse (*Mus musculus*) and chiken (*Gallus gallus*).

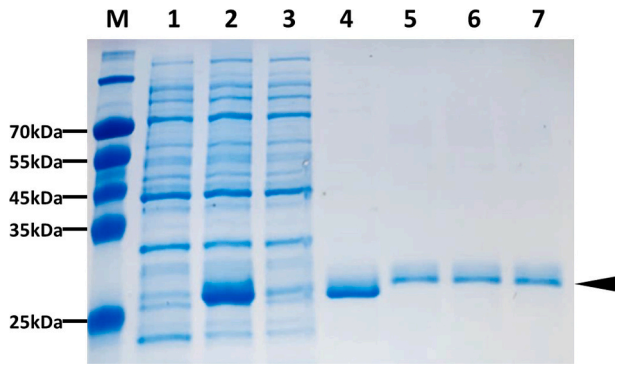

Fig. 2. SDS-PAGE analysis of recombinant Activin B in Japanese eel (M: Marker; lane 1: Total protein in the *E. coli* before induction; lane 2: Total protein in the *E. coli* after induction; lane 3: Protein in the precipitate after induction; lane 4: Protein in the supernatant after induction; lane 5–7: Recombinant Activin B after washing and refolding (26.4 kDa)).

groups had entered the vitellogenic stage, but there was no obvious difference between them (Fig. 3A). Measurement of serum E2 levels revealed that the treatment group exhibited significantly higher E2 concentrations compared to the control group ($p < 0.001$; Fig. 3B), which suggests that Activin B administration promotes E2 production during the ovarian developmental processes in Japanese eel.

3.4. Transcriptomic analysis reveals Activin B-mediated regulation of ovarian development in Japanese eel

To investigate the molecular mechanisms by which Activin B regulates ovarian development, transcriptomic analysis was performed on ovarian samples from both the Activin B-treated and control groups. High-throughput sequencing generated a total of 382,137,778 raw reads. After quality control and filtering, 368,707,628 clean reads were obtained. The average Q20 and Q30 scores were 98.85 % and 96.81 %, respectively, indicating high sequencing quality (Table S2).

Principal component analysis (PCA) revealed a clear separation between the Activin B-treated and control groups, indicating good classification and consistency among all eight biological replicates (Fig. 4A). A visual overview of DEGs is shown in Fig. 4B. As illustrated in Fig. 4C, a total of 1023 DEGs were identified, including 612 upregulated and 411 downregulated genes in the treatment group compared to the control.

To further explore the biological significance of these DEGs, GO and KEGG enrichment analyses were performed. Functional enrichment analyses revealed that the DEGs were broadly involved in processes related to signal transduction, transcriptional regulation, protein modification and transport, and cell cycle progression. At the cellular component level, many DEGs were associated with intracellular structures such as membranes, cytoskeleton, and organelles. In terms of molecular functions, enrichment was observed in categories related to enzyme activity, protein binding, and kinase-mediated phosphorylation, reflecting widespread regulatory potential (Fig. 4D). Consistently, KEGG pathway analysis highlighted significant enrichment in pathways governing protein processing, intracellular transport, cytoskeletal dynamics, and cell–cell interactions, along with several classical signaling cascades including MAPK, mTOR, and FoxO. Furthermore, metabolic pathways related to amino acids, lipids, and carbohydrates were also enriched, suggesting that Activin B regulates ovarian development through a coordinated network of signaling pathways and metabolic processes (Fig. 4E). As shown in Fig. 4F, the Sankey bubble plot revealed that DEGs were enriched in multiple signaling pathways closely related to ovarian development, with representative genes mapped to each pathway. Above results indicate that Activin B regulates ovarian development at the transcriptional level by modulating multiple biological processes and signaling pathways.

3.5. Untargeted metabolomic profiling reveals Activin B–regulated changes in ovarian metabolism

To further investigate the physiological changes associated with Activin B regulation of ovarian development, untargeted metabolomic analysis was performed on ovarian samples from both groups. PLS-DA revealed a distinct separation between the Activin B-treated and control groups, indicating differing metabolic states between the two groups (Fig. 5A). Among all the identified metabolites, the top three classes were Lipids and lipid-like molecules, Organic acids and derivatives, and Organoheterocyclic compounds, which accounted for 28.76 %, 18.59 %, and 18.43 % of the total metabolites, respectively (Fig. 5B).

A total of 553 DMs were identified between the two groups (Table S3), including 261 upregulated and 292 downregulated compounds (Fig. 5C, D). Among these, several estrogen-related steroids were detected, such as 19-Nortestosterone, Quinestrol, and 17-[(Benzylamino)methyl] estra-1,3,5(10)-triene-3,17β-diol, suggesting a potential link between Activin B treatment and steroid hormone metabolism. KEGG enrichment analysis revealed that the DMs were involved in purine, glycerophospholipid metabolism, biosynthesis of unsaturated fatty acids and fatty acid biosynthesis (Fig. 5E). These pathways are closely associated with cellular energy balance, membrane remodeling, and steroid hormone biosynthesis, all of which are essential for follicular development and ovarian function.

3.6. Integrated transcriptomic and metabolomic analysis reveals coordinated pathways regulated by Activin B

To elucidate the correlation between DEGs and DMs during Activin B–induced ovarian development, Pearson correlation analysis was performed. As shown in the correlation heatmap (Fig. 6A), several DEGs

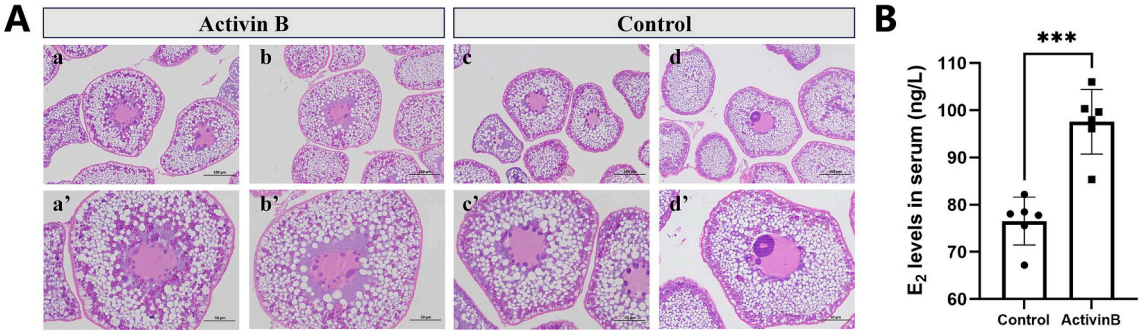

Fig. 3. (A) Representative ovarian sections from recombinant Activin B-treated and control groups stained with H&E. a-d, scale bar = 100 μm; a'-d' (represent magnified views of the corresponding regions in panels a–d), scale bar = 50 μm. (B) Comparison of estradiol (E2) levels between groups. Values represent mean ± SEM. $p < 0.05$.

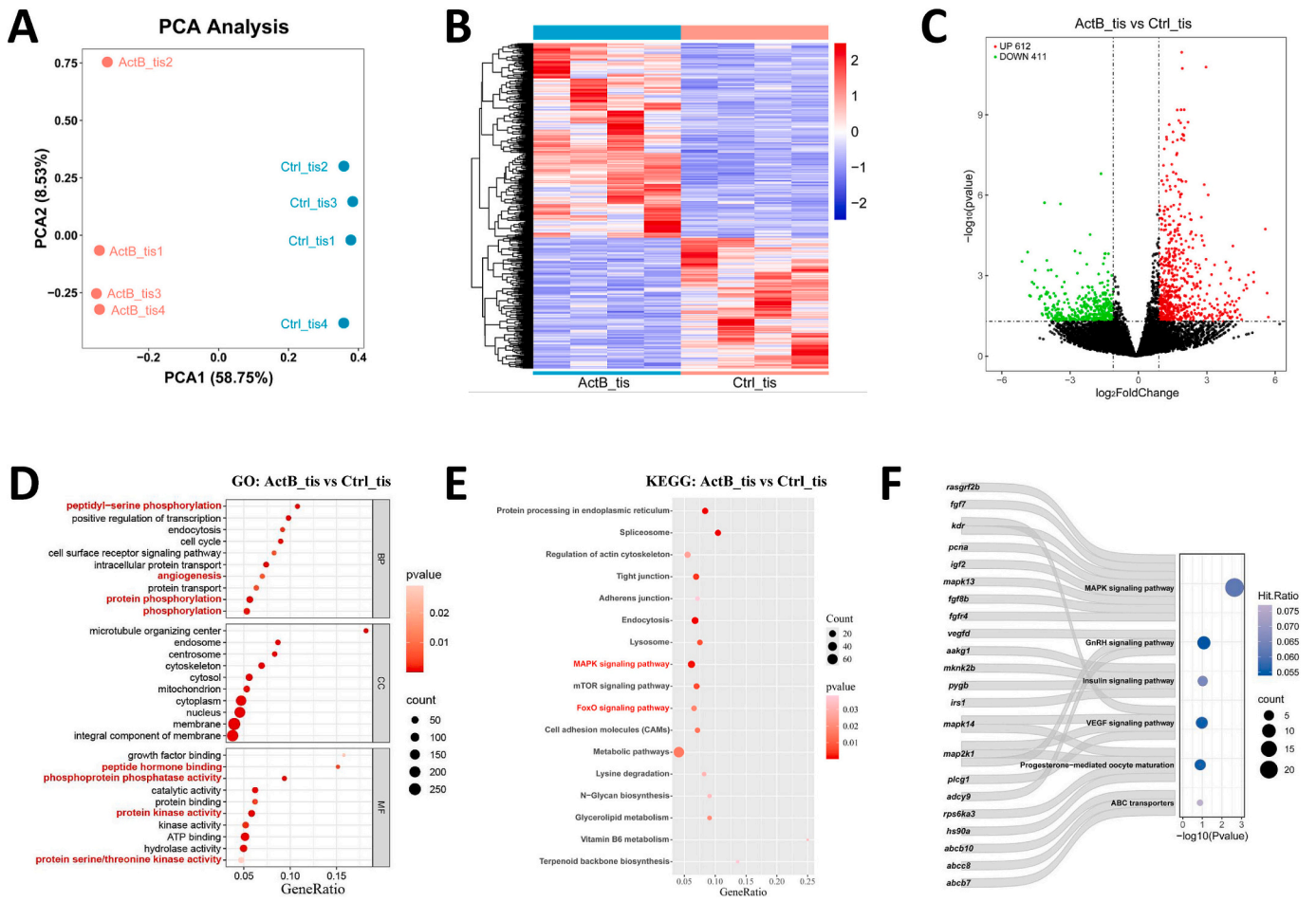

Fig. 4. (A) PCA of the eight libraries in the recombinant Activin B-treated and control groups. (B) The expression values of the DEGs in the recombinant Activin B-treated and control groups are presented in a heat map (red: upregulated, blue: downregulated). (C) Volcano plot of DEGs between recombinant Activin B-treated and control groups (red: upregulated, green: downregulated). (D) GO terms enriched among DEGs between recombinant Activin B-treated and control groups. (E) KEGG pathways enriched among DEGs between recombinant Activin B-treated and control groups. (F) DEGs in several reproduction-related signaling pathways.

exhibited significant positive or negative correlations with specific DMs, indicating potential regulatory relationships between gene expression and metabolic alterations. In addition, transcriptomic and metabolomic datasets were integrated based on shared KEGG pathways. Joint enrichment analysis identified several commonly affected pathways, including biosynthesis of unsaturated fatty acids, glycerophospholipid metabolism, GnRH signaling pathway, fatty acid biosynthesis and degradation, and biosynthesis of amino acids (Fig. 6B). These shared pathways highlight coordinated regulation at both the transcriptional and metabolic levels during Activin B–regulated ovarian development.

4. Discussion

An increasing number of studies have demonstrated that Activin B plays a pivotal role in ovarian development in teleosts [13,20,21]. In the present study, we preliminarily investigated the biological function of Activin B during ovarian development in Japanese eel. The ORF of Japanese eel Activin B is 1182 bp in length and encodes a protein of 393 aa, consisting of a 20-amino-acid signal peptide, a 258-amino-acid propeptide, and a 115-amino-acid mature peptide. Phylogenetic analysis and multiple sequence alignment revealed that Activin B is evolutionarily conserved across species, consistent with known evolutionary relationships. The Activin B protein of Japanese eel exhibits typical structural features of the TGF-β superfamily, including a distinct signal peptide, a relatively conserved mature region, and a characteristic pattern of cysteine residues. The mature peptide region contains conserved cysteine residues that are critical for the formation of intra-chain and interchain disulfide bonds, which stabilize the dimeric structure of the bioactive protein. Additionally, the conserved C-terminal region of the mature domain is responsible for interaction with type II Activin receptors, triggering downstream Smad2/3 signaling cascades [22–24]. Together, these structural features ensure that Activin B can function as a potent paracrine or autocrine regulator during ovarian development.

Artificial reproduction of Japanese eel remains a major challenge in aquaculture. Currently, ovarian development in captive female eels is primarily induced by repeated injections of pituitary extract. In this study, we investigated the potential biological function of recombinant Activin B in ovarian development by co-injecting it alongside a gonadotropin analog in adult females. Our aim was to investigate the regulatory role of Activin B in ovarian development of the Japanese eel and to elucidate its potential mechanisms, with the expectation of providing feasible insights to support artificial reproduction of this species.

Serum hormone measurements showed that E2 concentrations were significantly higher in the Activin B-treated group than in the controls, indicating that Activin B can promote steroid hormone production to some extent. Similar findings have been reported in zebrafish, where Activin has been shown to regulate steroidogenesis and follicular development, supporting the conclusions of our study [21,25]. Nevertheless, the absence of clear morphological differences in follicular development between treated and control groups suggests that Activin B does not directly drive vitellogenin uptake by oocytes.

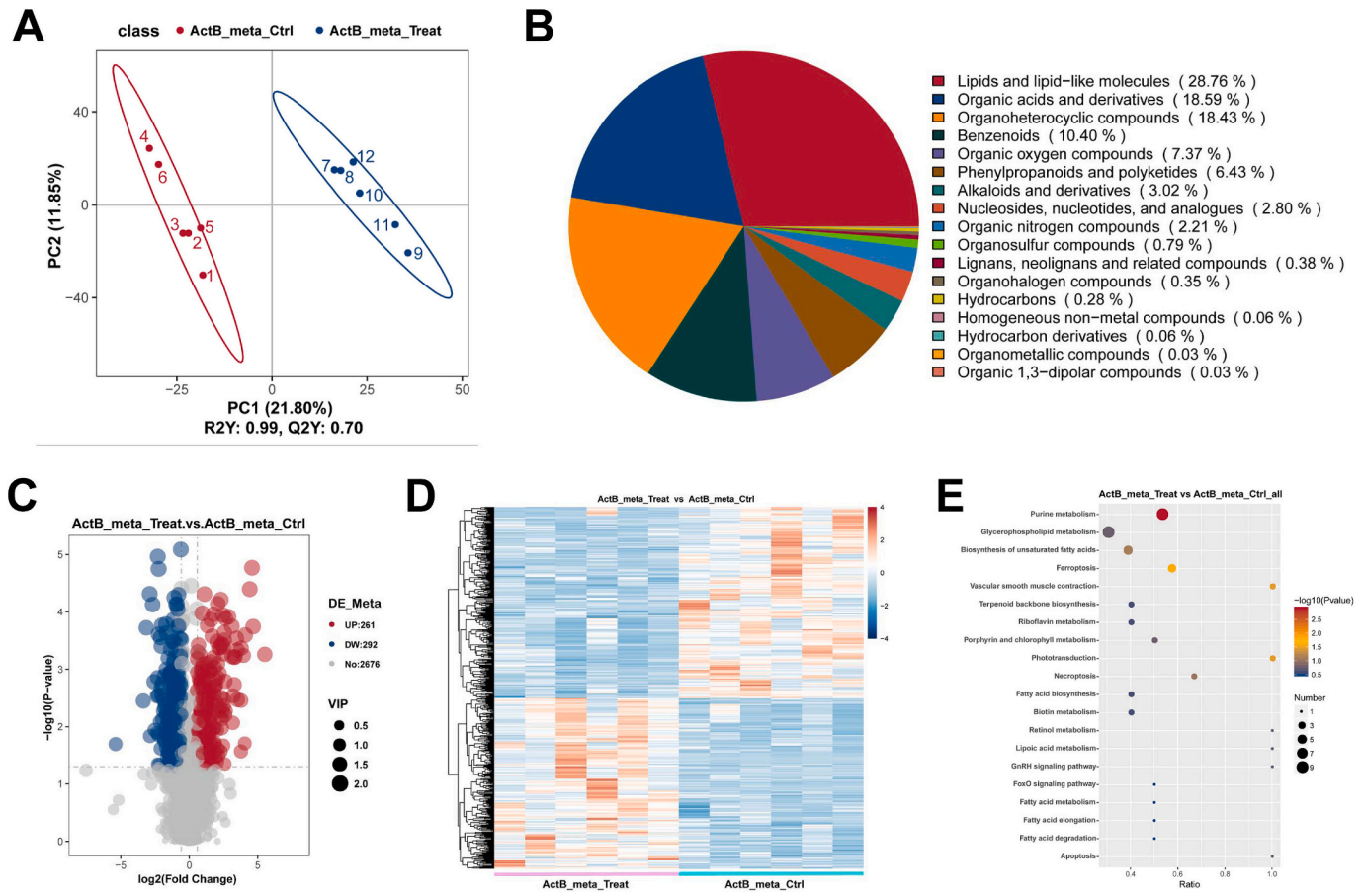

Fig. 5. (A) PLS-DA score plot showing separation between recombinant Activin B-treated and control groups. (B) The composition of the metabolites based on the databases. (C) The volcano plot of the metabolite peaks. (D) Heatmap of representative DMs between recombinant Activin B-treated and control groups. (E) KEGG pathway enrichment of DMs between recombinant Activin B-treated and control groups.

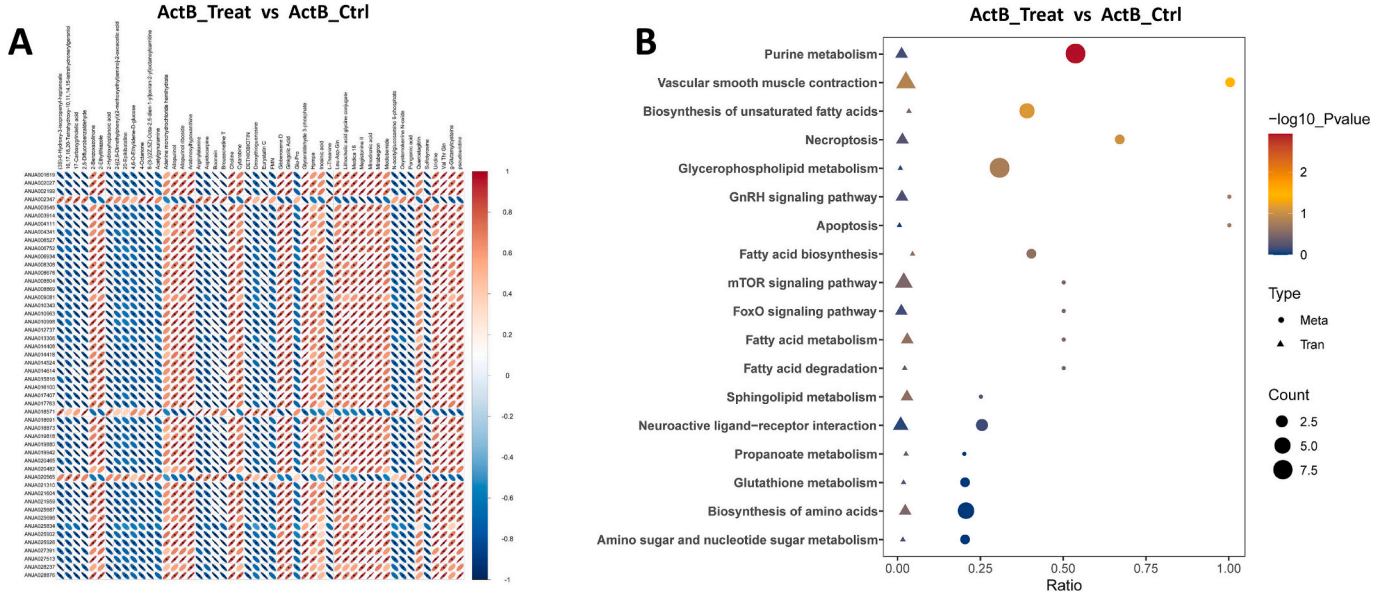

Fig. 6. (A) Correlation heatmap showing Pearson r values between selected DEGs (top 50) and DMs (top 50). Blue–red gradient indicates correlation strength; asterisks indicate statistical significance ($p < 0.05$). (B) Joint KEGG pathway enrichment analysis of DEGs and DMs.

To investigate the molecular mechanisms by which Activin B regulates ovarian development in Japanese eel, integrative bioinformatic analyses were conducted at both the transcriptomic and metabolomic levels. GO and KEGG enrichment analyses revealed that DEGs were significantly enriched in multiple signaling pathways related to Activin signaling and ovarian development, suggesting that Activin B may exert

its regulatory effects through coordinated activation of reproductive and hormone-related gene networks. GO enrichment analysis revealed significant involvement of signal transduction–related terms, including protein kinase activity, protein phosphorylation, and hormone binding. These findings indicate that Activin B likely initiates downstream signaling cascades to regulate ovarian development, which possibly including the cooperative interactions with parallel signaling cascades. In vertebrates, Activin B primarily signals through the Smad2/3-dependent TGF-β pathway, which involves binding to type II and type I Activin receptors and subsequent phosphorylation of Smad proteins [22]. These activated Smads form complexes with Smad4 and translocate to the nucleus, where they regulate the transcription of target genes involved in steroidogenesis and follicular development [26]. In addition to the canonical Smad-dependent signaling pathway, non-canonical pathways such as MAPK, PI3K-Akt, and ERK may also be activated concurrently or synergistically with Smad signaling. Increasing evidence suggests that members of the TGF-β superfamily, including various regulatory ligands, can exert their biological effects through the activation of multiple signaling cascades in parallel [27–29]. This has been demonstrated in studies of some teleosts, where members of the TGF-β family have been shown to activate both Smad-dependent and Smad-independent pathways to regulate complex physiological processes [30,31]. Such a mechanism may also allow Activin B to fine-tune ovarian development through the integration of diverse intracellular signals. In our study, the MAPK signaling pathway was significantly enriched in the KEGG analysis, suggesting that Activin B may activate both Smad-dependent and MAPK-mediated signaling pathways to coordinately regulate ovarian development in Japanese eel. Above results indicate that Activin B functions through a combination of canonical and non-canonical mechanisms to fine-tune gene expression and physiological responses during ovarian development in Japanese eel.

A complex vascular network is essential for ovarian development, ensuring the delivery of oxygen, hormones, and nutrients, as well as the removal of metabolic waste products [32–34]. In this study, GO functional analysis revealed significant enrichment of the angiogenesis, suggesting that Activin B may regulate blood vessel formation during the ovarian development in Japanese eel. Such vascular remodeling could facilitate adequate nutrient supply and metabolic exchange, thereby regulating oocyte growth at this critical stage of development. Similar observations have been reported in other vertebrates, where members of the TGF-β superfamily, including Activins, have been shown to stimulate endothelial cell proliferation, migration, and capillary-like structure formation, thereby enhancing angiogenesis within reproductive tissues [35,36]. These parallels support the hypothesis that, in teleosts, Activin B may exert a conserved regulated-angiogenic function to optimize the microenvironment for oocyte development.

In the present study, KEGG enrichment analysis revealed significant representation of the FoxO signaling pathway among the DEGs, suggesting its potential involvement in Activin B–mediated ovarian development in Japanese eel. Members of the Fox family, particularly FoxL2 and FoxO transcription factors, are known to play pivotal roles in regulating folliculogenesis, steroidogenesis, and oocyte maturation in vertebrates [37,38]. The enrichment of the FoxO signaling pathway in our data implies that Activin B may exert part of its effects through transcriptional regulation mediated by FoxO factors. Such interactions may fine-tune steroid hormone biosynthesis, maintain follicle health, and coordinate oocyte growth during ovarian development.

In addition to the pathways described above, several DEGs were also enriched in other signaling pathways that are closely linked to ovarian development, including the GnRH signaling pathway, insulin signaling pathway, VEGF signaling pathway, progesterone-mediated oocyte maturation, and ATP-binding cassette (ABC) transporters. GnRH signaling acts at the top of the reproductive endocrine hierarchy, driving gonadotropin release from the pituitary and thereby influencing downstream processes such as steroid hormone synthesis and gametogenesis [39]. The Insulin signaling, often through cross-talk with PI3K/Akt and MAPK pathways, supports follicle growth, granulosa cell proliferation, and the regulation of steroid production [40,41], while VEGF signaling is indispensable for the extensive angiogenesis that accompanies ovarian development, ensuring that developing oocytes receive adequate oxygen, nutrients, and hormonal cues [42,43]. Meanwhile, ABC transporters, are involved in the transmembrane movement of lipids, cholesterol, and other substrates, potentially influencing the supply of essential molecules for steroid biosynthesis and follicular growth [44]. The enrichment of these pathways in our data suggests that Activin B may regulate Japanese eel ovarian development via a complex, multi-pathway network that integrates endocrine regulation, local signaling, nutrient transport, and angiogenesis.

Notably, both the metabolomic analysis and the integrated transcriptome–metabolome analysis also revealed enrichment of the GnRH and FoxO signaling pathways, providing further support for the transcriptomic findings. The recurrence of these pathways across different omics layers reinforces their potential importance in mediating the effects of Activin B on ovarian development. Furthermore, the untargeted metabolomic analysis revealed pronounced alterations in lipid and amino acid metabolism, with notable changes in pathways related to steroid hormone metabolites, energy metabolism, and the biosynthesis and catabolism of several amino acids. These metabolic shifts align well with integrated multi-omics findings, offering additional evidence that Activin B may influence ovarian development in Japanese eel through multiple, interconnected processes that encompass endocrine regulation, nutrient metabolism, and energy homeostasis. Taken together, the integrated transcriptomic and metabolomic analyses provide comprehensive insights into the molecular mechanisms underlying Activin B–regulated ovarian development in Japanese eel. Transcriptome profiling revealed that Activin B regulates the expression of genes involved in steroid biosynthesis, oocyte development, and hormone signaling pathways. In parallel, metabolomic analysis identified significant alterations in metabolites associated with lipid metabolism, nucleotide turnover, and steroid hormone pathways, including several estrogen-like compounds. The coordinated enrichment of key biological processes—such as steroidogenesis, purine metabolism, and unsaturated fatty acid biosynthesis—highlights the dual regulatory role of Activin B at both the transcriptional and metabolic levels. These findings suggest that Activin B regulates ovarian development not only by activating hormone-related gene networks but also by inducing metabolic reprogramming to meet the energetic and biosynthetic demands.

The present study investigated the potential role and molecular mechanisms of Activin B in ovarian development of the Japanese eel primarily through intramuscular injection, combined with transcriptomic and metabolomic analyses. However, key production-related parameters such as fertilization rate, hatching rate, and larval survival were not assessed. In future studies, we aim to improve the experimental design by incorporating these reproductive performance indicators, thereby providing stronger evidence and more practical support for the artificial propagation of Japanese eel.

5. Conclusion

This study provides new insights into the role of Activin B in ovarian development of the Japanese eel. Previous evidence from mammals and teleosts has shown that Activin B is a critical regulator of gonadal function through endocrine and intra-gonadal pathways. Here, we cloned and characterized the *inhbb* gene, expressed recombinant Activin B protein, and demonstrated its biological function in vivo. Functional analyses revealed that Activin B significantly increased serum estradiol levels and modulated transcriptional and metabolic profiles. Integrated transcriptomic and metabolomic analyses further indicated that Activin B exerts its effects through both canonical (Smad) and non-canonical (MAPK) cascades, together with reproductive signaling pathways such as GnRH, insulin, and VEGF. These networks converge on lipid

metabolism, amino acid metabolism, and steroidogenesis, thereby regulating ovarian development. Collectively, this study provides preliminary insights into the potential roles and mechanisms of Activin B in regulating ovarian development in Japanese eel, offering a theoretical basis for artificial reproduction.

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CRediT authorship contribution statement

Chenpeng Zuo: Writing – original draft, Data curation. **Xuanhan Zhang:** Investigation, Data curation. **Xiao Jing:** Data curation. **Yuting Ci:** Data curation. **Ziyi Zhao:** Data curation. **Ming Liu:** Data curation. **Yun Li:** Resources. **Haishen Wen:** Resources, Conceptualization. **Xin Qi:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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