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Adaptive evolution of *BMP4* as a potential mechanism for flipper forelimb changes in cetaceans

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ABSTRACT

Vertebrate limbs have undergone profound morphological diversification, enabling adaptations to a broad spectrum of ecological niches. In marine mammals, the evolution of highly specialized flipper-like forelimbs represents a profound structural transformation associated with aquatic habitats. This adaptation has been hypothesized to result, in part, from the inhibition of interphalangeal cell apoptosis during limb development, although the underlying genetic mechanism remains poorly understood. This study investigated the evolutionary dynamics and functional consequences of three key bone morphogenetic protein genes, *BMP2*, *BMP4*, and *BMP7*, which regulate apoptosis in interphalangeal mesenchymal stromal cells during embryonic limb development to ensure proper differentiation of interphalangeal tissues. Comparative genomic analysis revealed significantly accelerated evolution for *BMP4* and *BMP7* in the cetacean ancestral lineage, with two positively selected sites (V79I and H247R) involved in cetacean-specific amino acid substitutions located in the TGF- β propeptide functional domain in *BMP4*. *In vitro* assays confirmed that cetacean-specific *BMP4* mutations significantly disrupted normal cell apoptosis and proliferation and altered the transcription and protein expression of downstream apoptosis-related factors, including cytochrome c (Cyt c), BCL2 associated X, and B-cell lymphoma 2, within the BMP signaling pathway. The significant influence of *BMP4* mutations on apoptotic inhibition highlights a potential role in the development of limb bud mesenchymal tissue and the emergence of the flipper forelimb phenotype in cetaceans.

Keywords: Cetaceans; Flipper limb; *BMP4*; Functional change

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INTRODUCTION

The emergence and evolutionary refinement of vertebrate limbs represent an important innovation facilitating the transition from aquatic to terrestrial environments and providing the foundation for expanded survival and reproductive strategies (Coates, 1994). Since their origin in the Ordovician period, vertebrate limbs have undergone extensive morphological diversification, supporting adaptive radiation into a wide range of ecological niches (Shubin et al., 1997). In mammals, this diversification has produced highly specialized limb forms and functions that reflect lineage-specific ecological strategies (Bi et al., 2023; Eckalbar et al., 2016; Rothier et al., 2023; Royle et al., 2021; Saxena et al., 2017; Sears et al., 2018; Young & Tabin, 2017). As a reliable indicator of environmental adaptation, limbs have become a central focus in evolutionary developmental biology.

Among mammals, cetaceans exemplify secondary adaptation to aquatic habitats, characterized by radical reorganization of tissue architecture and organ systems (Huelsmann et al., 2019; Qi & Shi, 2016). Originating from terrestrial artiodactyl ancestors approximately 55 million years ago (Ma), cetaceans have undergone profound transformations in their limb morphology (Thewissen et al., 2006). Unlike their fully degenerated hindlimbs, cetacean forelimbs have been transformed into broad, rigid flippers that enable precise maneuverability, propulsion control, and stabilization in the marine environment (Bejder & Hall, 2002; Cooper et al., 2007). Despite the remarkable nature of these morphological innovations, few studies have focused on the evolution of flippers in cetaceans and the genetic underpinnings of this phenotypic modification remain poorly resolved. Early embryological analyses of the morphology and

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developmental biology of the pantropical spotted dolphin (*Stenella attenuata*) identified the early termination of key limb-patterning genes (e.g., *FGF8* and *SHH*) in developing whale limb buds (Richardson & Oelschläger, 2002). Subsequently, Cooper et al. (2018) discovered the presence of supernumerary phalanges with interphalangeal tissues in cetacean forelimbs, alongside altered spatiotemporal expression of apoptotic and morphogenetic regulators such as BMP2, BMP4, Gremlin, and FGF8. These findings suggest that modulation of the bone morphogenetic protein (BMP) signaling axis may play a central role in the retention of interphalangeal tissue and flipper morphogenesis. More recently, Telizhenko et al. (2024) identified relaxed evolution in certain limb development-regulating genes in cetaceans.

The morphogenesis of vertebrate limbs requires a dynamic balance between cellular differentiation and apoptosis to ensure the proper formation of distal finger architecture (Duboc & Logan, 2011). During early limb bud development, interdigit tissue components establish complex interactions, coordinating cell fate and tissue patterning through the secretion of diverse regulatory molecules. This signaling network governs key processes, including apoptosis, cell cycle arrest, and extracellular matrix remodeling within the interphalangeal region (Lorda-Diez et al., 2015; Montero & Hurlé, 2010; Montero et al., 2021; Varga & Varga, 2022). The BMP signaling pathway, a branch of the transforming growth factor beta (TGF- β) superfamily, plays a crucial role in phalangeal joint development and interphalangeal tissue apoptosis (Pajni-Underwood et al., 2007; Robert, 2007). Key components of this pathway, including *BMP2*, *BMP4*, and *BMP7*, are prominently expressed in the apical ectodermal ridge (AER), a critical signaling center during limb bud development, and within interstitial regions of the interphalangeal space (Choi et al., 2012; Delgado & Torres, 2017). These BMPs bind to BMP receptors (Bmpr1a/1b) to modulate fibroblast growth factor (FGF) signaling in the AER, coordinating morphogenetic processes that define distal digit architecture and orchestrate the spatial patterning of digit formation (Bandyopadhyay et al., 2006). Functional genetic studies in mice have demonstrated that conditional knockout of *BMP2*, *BMP4*, and *BMP7*—individually or in combination—leads to AER disruption, polydactyly, and impaired interphalangeal cell proliferation and apoptosis (Maatouk et al., 2009; Salazar et al., 2016). In some taxa, such as bats, BMP-regulated interdigital apoptosis is selectively attenuated, resulting in the evolutionary retention of webbing and wing membrane structures (Cooper et al., 2012; Eckalbar et al., 2016; Weatherbee et al., 2006). These observations underscore the central role of BMP signaling in regulating interphalangeal cell apoptosis and the patterning of digit morphology.

Despite significant advancements in our understanding of the molecular mechanisms governing interphalangeal apoptosis in mice, chickens, and waterfowl species (Montero et al., 2021), the emergence of the cetacean forelimb flipper represents an extreme morphological departure from typical mammalian limb architecture, characterized by the retention of interphalangeal tissue. However, the molecular basis of this transformation remains poorly defined. This study investigated the evolutionary trajectories of *BMP2*, *BMP4*, and *BMP7*, key regulators of appendage development in mammals. Comparative bioinformatic analyses, coupled with *in vitro* validation, identified lineage-specific acceleration and unique

amino acid substitutions in cetacean *BMP4* that inhibited apoptosis, thereby facilitating the retention of interphalangeal tissue and the emergence of the flipper phenotype. These findings provide novel mechanistic insights into cetacean appendage evolution and the molecular drivers of secondary aquatic adaptation.

MATERIALS AND METHODS

Multiple sequence alignment and phylogenetic analysis

A total of 86 mammals, including 13 cetacean species, were selected for comparative analysis (Supplementary Table S1). Coding sequences of BMPs (*BMP2*, *BMP4*, and *BMP7*) were retrieved from the National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>) and OrthoMaM v.12a databases (<https://orthomam.mbb.cnrs.fr/>). Sequence alignment was performed using the PRANK plugin in FasParserv.2.13.0 (<https://github.com/Sun-Yanbo/FasParser/releases>) (Sun, 2018), followed by filtering using Gblocks with the parameters “-t=c, -b5=h” (Castresana, 2000). The aligned *BMP2*, *BMP4*, and *BMP7* sequences were concatenated using the Concatenate Sequence program in PhyloSuite (v.1.2.2) with default parameters (Zhang et al., 2020). Optimal partitioning strategies and model selection were determined based on corrected Akaike Information Criterion (AICc) using PartitionFinder2. A maximum-likelihood (ML) phylogenetic tree was constructed using IQ-TREE based on the optimal model, applying ultrafast bootstrap analysis to assess node support. Final tree visualization was performed using FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Molecular evolutionary analysis of BMPs

Selection pressure analysis was conducted using the Codeml program in PAML v.4.9 (Yang, 2007). The nonsynonymous (d_N) to synonymous (d_S) substitution ratio ($\omega=d_N/d_S$) was calculated to infer evolutionary constraints, with $\omega>1$, $0<\omega<1$, and $\omega=1$ indicating positive, purifying, and natural evolution, respectively. Phylogenetic topology was based on McGowen et al. (2020) and calibrated using TimeTree (<http://www.timetree.org/>) (Supplementary Figure S1). Four models were tested: one-ratio (model=0, NSsites=0), free-ratio (model=1, NSsites=0), two-ratio (model=2, NSsites=0), and branch-site models (Ma and Ma0). In the branch-site model, the null hypothesis (Ma0, model=2, NSsites=2, fix_omega=1, omega=1) and alternative hypothesis (Ma, model=2, NSsites=2, fix_omega=0) were compared, with the cetacean ancestral branch designated as the foreground branch to assess selective pressure. Significant differences between ω values were assessed using the likelihood ratio test (LRT) and chi-square test. Positively selected sites were identified using Bayes empirical Bayes (BEB) with posterior probabilities (PP) ≥ 0.8 (Yang & Nielsen, 2002).

Cetacean-specific site identification and protein structure analysis

Cetacean-specific amino acid substitutions in BMPs were identified using FasParser v.2.13.0. Functional characterization of these substitutions was performed using the aaml program in PAML v.4.9 with the following parameters: “aaRatefile=jones.dat, model=3, NSsites=0”. Physicochemical properties and functional annotations of the cetacean-specific sites were assessed using the UniProt database (<https://www.uniprot.org/>). Structural impacts of these mutations were evaluated by generating a three-

dimensional (3D) model, using the mouse BMP sequences as a reference, via the Swiss Model platform (<https://swissmodel.expasy.org/>), selecting models with the highest GMQE scores. Cetacean-specific mutations were incorporated into the model, and visualization was performed using Ezmol (<http://www.sbg.bio.ic.ac.uk/ezmol>).

Plasmid construction and mutation

Full-length cDNAs of murine BMPs (mBMPs) and dolphin BMPs (dBMPs) were amplified and cloned into the pcDNA3.1 expression vector using KpnI and BamHI restriction sites. Cetacean-specific mutant plasmids of BMPs (mut-mBMPs) were constructed using a QuickMutation™ Site-Directed Mutagenesis Kit (D0206M, Beyotime Biotechnology, China). All constructs were verified through Sanger sequencing. All primer sequences are listed in Supplementary Table S2.

Cell culture and transfection

To further validate the bioinformatic results, the pcDNA3.1-mBMP4, dBMP4, and mut-mBMP4 plasmids were constructed and transiently overexpressed with a C-terminal 3×FLAG tag in the mouse fibroblast cell line (NIH3T3). NIH3T3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum at 37°C and 5% CO₂. When cells reached 50%–60% confluency, BMPs and pcDNA3.1 fusion constructs were transiently transfected using Lipofectamine™ 3000 transfection reagent (Invitrogen, USA), following the manufacturer's protocols.

Apoptosis assay via flow cytometry

Apoptotic cell populations were quantified using flow cytometry with Annexin V/Propidium Iodide (PI) double staining, following the manufacturer's protocols (KGA1030-50, KeyGEN BioTECH, China). NIH3T3 cells (1×10^6) were seeded in 6-well plates and treated for 15 h with apoptosis inducers (Apoptida: Apobid=1 500:1) (Beyotime Biotechnology, China). After treatment, the cells were harvested and resuspended in 500 µL of binding buffer, with sequential addition of 5 µL of Annexin V and an equal volume PI and subsequent incubation in the dark for 15 min. Apoptotic cells were quantified using a FACSCalibur cytometer (BD Biosciences, USA), and data were analyzed using FlowJo™ v.10.8 (BD Life Sciences, USA).

Cell proliferation assay

Cell proliferation was evaluated using a CCK-8 assay kit (A311-01, Vazyme, China). Transfected NIH3T3 cells (4×10^3) were seeded in 96-well plates and cultured for 12 h at 37°C. Subsequently, 10 µL of CCK-8 solution was added to each well, followed by incubation for 12 h and an additional 2 h in the dark. Absorbance was measured at 450 nm using a microplate reader (BioTEK, USA).

Reverse transcription quantitative PCR (RT-qPCR)

To detect differences in transcriptional expression among plasmids, NIH3T3 cells were transfected with BMP4 expression constructs and seeded in 12-well plates at 37°C with 5% CO₂. After 48 h, total RNA was extracted using RNA Isolater Total RNA Extraction Reagent (R401, Vazyme, China) in accordance with the manufacturer's protocols and reverse-transcribed into cDNA using the HiScript III TR SuperMix for qPCR (+gDNA wiper) Reverse Transcription Kit (R323, Vazyme, China). qPCR was performed using SYBR qPCR Master Mix (Q712, Vazyme, China) with specific primers (Supplementary Table S3) on a Roche LightCycler® 480.

Relative gene expression was calculated using the $2^{-\Delta\Delta ct}$ method (Livak & Schmittgen, 2001). Statistical significance was assessed using unpaired Student's *t*-test across three biological replicates.

Western blotting

Western blot analysis was employed to assess protein expression related to BMP-mediated apoptotic signaling. NIH3T3 cells transfected in 6-well plates were harvested after 48 h and treated with cell lysate buffer (RIPA: PMSF=100:1 200 µL per well). Lysates were mixed with 5×loading buffer (1:5 ratio) at 95°C for 10 min for protein denaturation. Proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, P0012A, Beyotime Biotechnology, China), then transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 5% nonfat dry milk for 2 h, the membranes were incubated overnight at 4°C with primary antibodies FLAG (AE063, ABclonal, China) and β-Actin (AC038, ABclonal, China), followed by incubation with secondary antibodies (horseradish peroxidase (HRP) goat anti-rabbit IgG, AS014, ABclonal, China) for 1 h at room temperature. Signal detection was performed using a chemiluminescent imaging system, band intensities were quantified using ImageJ v.1.46r (Schneider et al., 2012), and data were plotted using GraphPad Prism v8.0 (USA, www.graphpad.com).

Statistical analysis

All data are expressed as mean±standard deviation (SD) from at least three independent experiments. Two-tailed Student's *t*-test was performed for statistical analysis, and all graphs were generated with GraphPad Prism v.8.0, with *P*<0.05 considered statistically significant.

RESULTS

Unique evolutionary dynamics of BMPs in cetaceans

To elucidate the evolutionary patterns of BMPs in marine mammals, phylogenetic relationships were reconstructed based on the coding sequences of *BMP2*, *BMP4*, and *BMP7* from 86 representative mammalian species. The phylogenetic relationships inferred from the three genes (Supplementary Figure S2) were largely congruent with previously reported topologies (Feigin et al., 2023). Branch model analyses revealed a significant acceleration in the evolutionary rate of *BMP4* within the cetacean ancestral lineage ($\omega=0.229$, *P*<0.01) (Supplementary Table S4 and Figure S3). Branch-site model analysis, with the cetacean ancestral lineage designated as the foreground branch, revealed significant evidence of positive selection in *BMP4*. Likelihood ratio testing between the Null ($\text{Ma}0$, $0<\omega_0<1$, $\omega_1=\omega_2=1$) and alternative models (Ma , $0<\omega_0<1$, $\omega_1=1$, $\omega_2\geq 1$) showed significant positive selection signals in *BMP4* within the cetacean ancestral lineage. In addition, several positive selection sites were detected by the BEB method, each with a posterior probability ≥ 0.8 (Supplementary Table S5).

Several cetacean-specific amino acid substitutions in BMPs were identified compared to other mammalian taxa. Notably, two specific amino acid substitution sites (I79V, H247R) in cetacean *BMP4* were identified as positively selected sites (Figure 1A; Supplementary Table S5). Structural modeling predicted that these mutations were localized within the TGF-β-propeptide functional domain (Figure 1B).

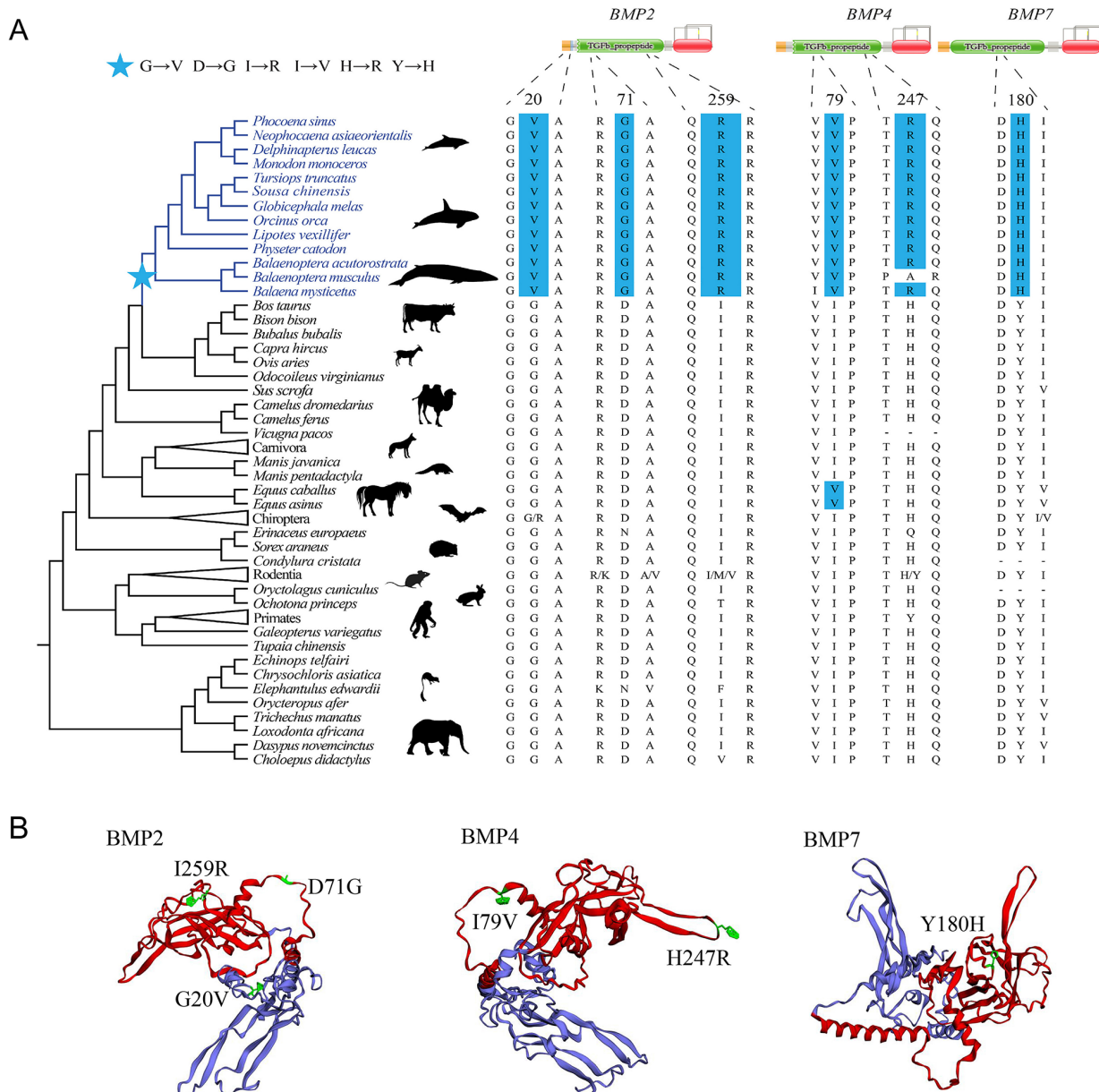


Figure 1 Specific amino acid alterations in cetaceans

A: Left: Phylogenetic tree of species in this study. Right: Blue boxes highlight lineage-specific mutations, all located within or adjacent to functional domains of the genes. B: 3D structural prediction of cetacean BMP proteins (BMP2, BMP4, and BMP7). Red represents TGF- β propeptide functional domain, green indicates cetacean-specific amino acid sites.

Cetacean-specific BMP4 mutations alter apoptosis and proliferation dynamics

Flow cytometry demonstrated a substantial reduction in apoptotic cell populations following transfection with dBMP4 (10.23%) compared to mBMP4 (17.99%). To further assess the functional impact of cetacean-specific substitutions, a mutant construct (mut-mBMP4) was generated. Notably, mut-mBMP4-transfected cells showed significantly lower apoptosis levels compared to the control group (mBMP4-transfected cells) (Figure 2A, B). Similarly, CCK8 assays revealed a significant reduction in cell proliferation in both dBMP4- and mut-mBMP4-expressing cells relative to mBMP4 controls ($P < 0.05$) (Figure 2C).

Cetacean-specific BMP4 and its mutations down-regulate intracellular apoptosis-related genes

The RT-qPCR results indicated that cetacean-specific *BMP4*

and its amino acid substitutions significantly down-regulated the mRNA expression levels of intracellular pro-apoptotic genes *Bax* and *Cyt c* (Figure 3A, C, D). Correspondingly, the transcriptional level of the anti-apoptotic gene *Bcl-2* was markedly up-regulated (Figure 3B). Western blot analysis further confirmed these observations, showing significant down-regulation of Bax and Cyt c protein levels in dBMP4-transfected cells (Figure 4).

In contrast, cetacean-specific mutations in BMP2 and BMP7 showed no significant changes in apoptosis (Supplementary Figure S4A, C), cell proliferation (Supplementary Figure S4B, D), or the mRNA expression of key intracellular apoptosis-related genes (Supplementary Figures S5, S6). Additionally, no significant changes were detected in BMP signaling protein levels (Supplementary Figures S7, S8). These findings suggest that mutations in cetacean BMP2 and BMP7 do not exert measurable functional effects at the cellular level.

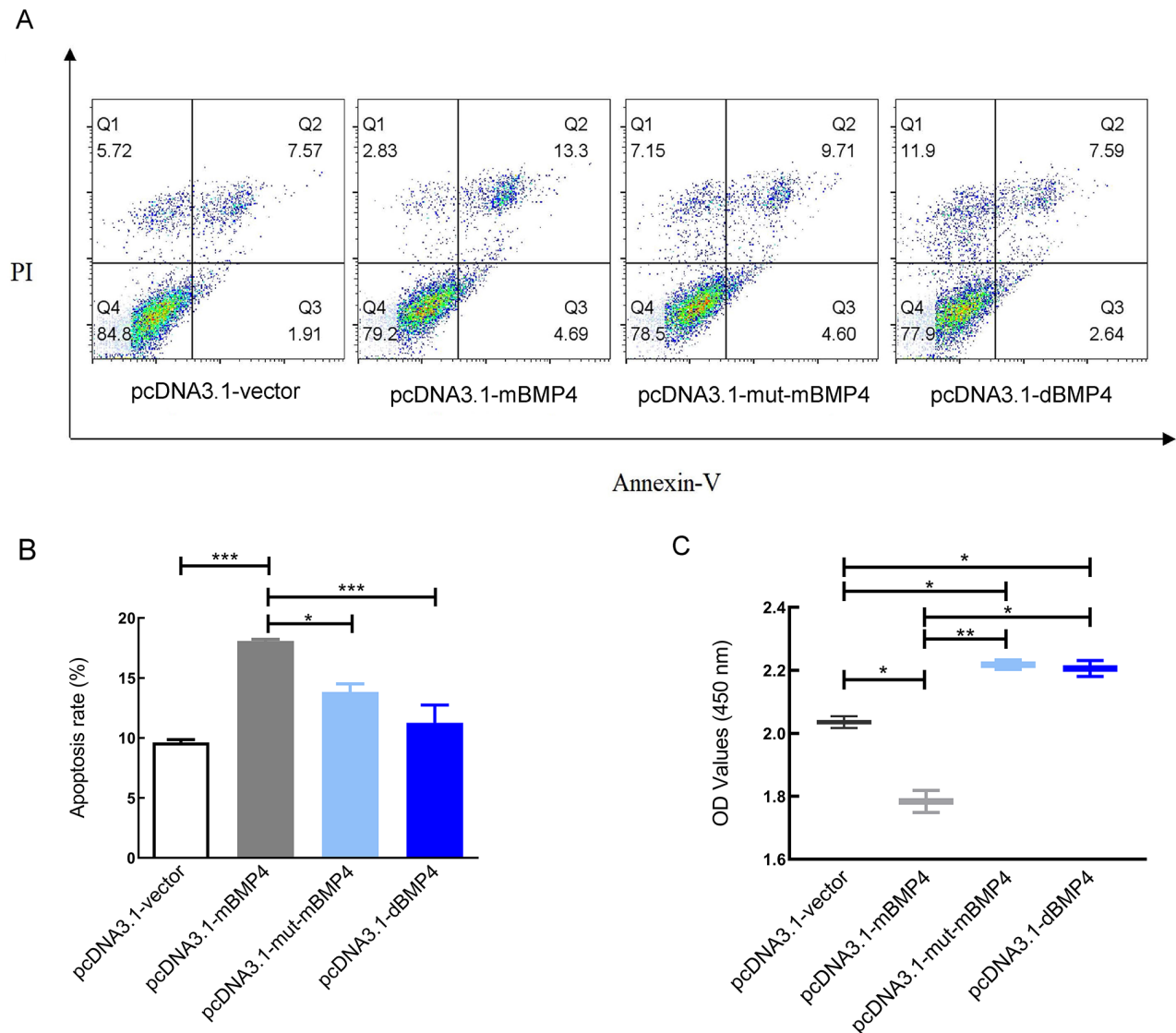


Figure 2 Assessment of apoptosis and proliferation in NIH3T3 cells overexpressing *BMP4* plasmids

A: Flow cytometry profiles of cells transfected with mBMP4, dBMP4, or mut-mBMP4. B: Quantification of apoptotic populations using Annexin V/PI dual staining, distinguishing live cells (Annexin V-/PI-), early apoptotic cells (Annexin V+/PI-), and late apoptotic cells (Annexin V+/PI+). C: CCK-8 assay measuring cell proliferation in BMP4-transfected NIH3T3 cells. Data are presented as mean $\pm$ SD, *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

DISCUSSION

Digit formation in vertebrate limb buds serves as a classic example of morphogenesis driven by programmed cell death (Zuzarte-Luis & Hurle, 2005). Apoptosis functions as a tightly regulated mechanism integrated with differentiation and proliferation to shape limb architecture at every developmental stage. In several amniote lineages, variations in apoptotic patterns during embryonic development have contributed to the emergence of specialized limb morphologies adapted for unique ecological demands (Montero et al., 2021). Among the molecular regulators orchestrating this process, BMPs, such as *BMP2*, *BMP4*, and *BMP7*, play critical roles in initiating apoptotic signaling within the ectodermal and mesodermal compartments of the AER, as demonstrated in transgenic mouse models (Lin & Zhang, 2020). This study integrated comparative genomic analysis with cellular functional assays to identify distinct evolutionary trajectories of *BMP2*, *BMP4*, and *BMP7* in cetaceans, pointing to their involvement in adaptations to secondary aquatic environments, particularly in the development of flipper forelimbs. Notably, cetacean-

specific evolutionary changes in *BMP4* were found to significantly disrupt normal patterns of cell proliferation and apoptosis, implicating these mutations in the inhibition of interdigital cell apoptosis during limb morphogenesis and the emergence of the cetacean flipper phenotype (Figure 5).

Previous developmental studies have established *BMP2*, *BMP4*, and *BMP7* as crucial regulators of limb development during embryogenesis, contributing to both early limb patterning and subsequent endochondral osteogenesis in cartilage (Grall et al., 2024). While *BMP2* and *BMP7* appear to act transiently during the initiation of interdigital cell death (Salas-Vidal et al., 2001), *BMP4* is thought to exert a more sustained regulatory influence within the AER mesoderm, modulating digit specification and patterning (Selever et al., 2004). Ectopic or dysregulated expression of these BMPs disrupts normal limb bud morphogenesis, as evidenced in bats, where altered expression patterns of *BMP2*, *BMP4*, and *BMP7* have contributed to aberrant interdigital apoptosis (Cooper et al., 2012). Comparative analysis of *cis*-regulatory elements (CREs) of key genes involved in appendage

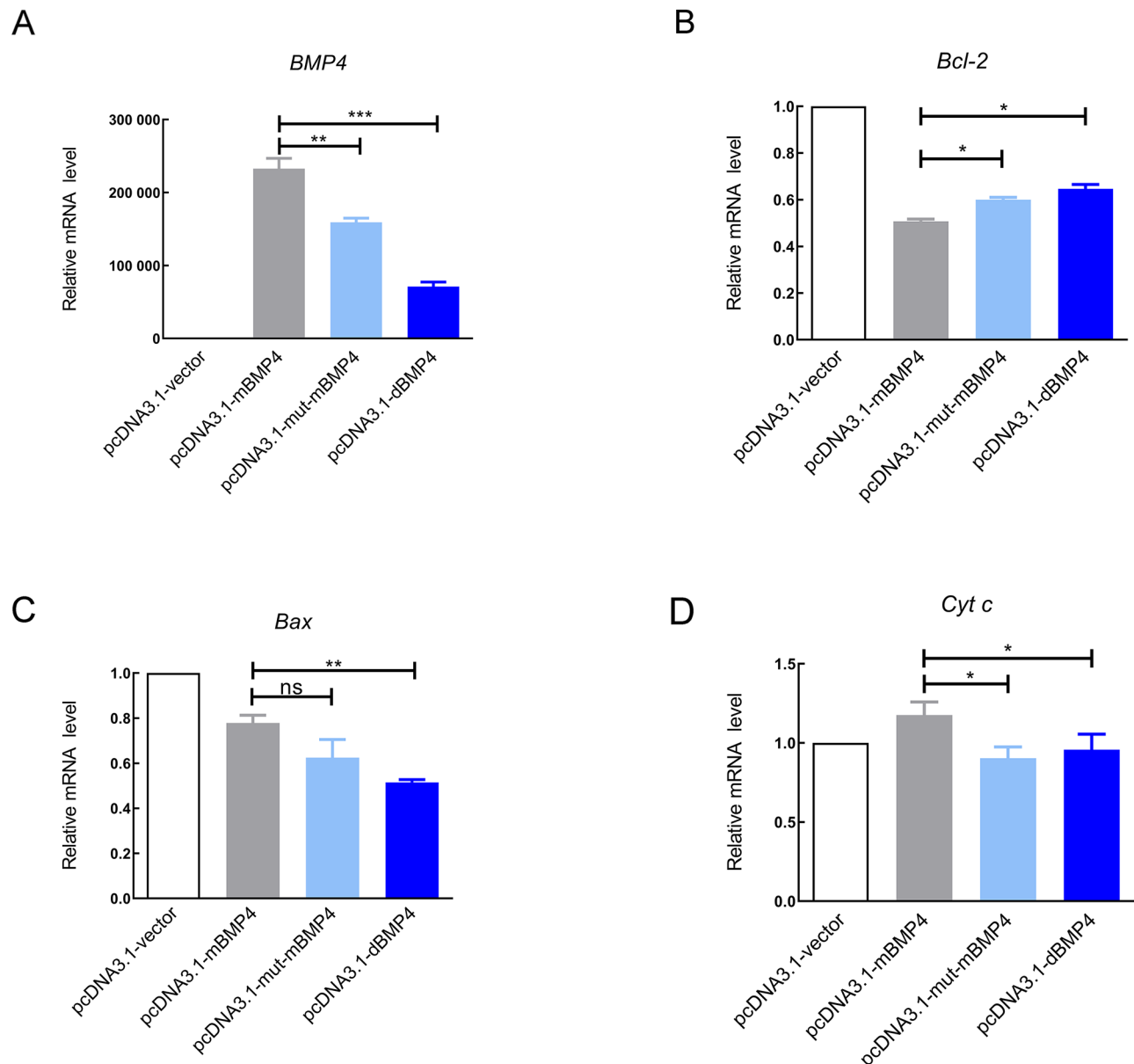


Figure 3 Transcriptional expression levels of *BMP4* in cetaceans and mutant constructs

A: Relative mRNA expression of *BMP4* in cells transfected with mBMP4, mut-mBMP4, and dBMP4 constructs. B–D: Expression levels of three candidate apoptotic genes in NIH3T3 cells transfected with vector, mBMP4, mut-mBMP4, and dBMP4. Data are presented as mean±SD, ns: Not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

development within the BMP signaling pathway in marine mammals revealed accelerated evolutionary signals in two CREs located near BMP genes (Sun et al., 2022), highlighting the evolutionary importance of BMPs in cetacean flipper morphology. Here, analysis of the evolutionary trajectories of *BMP2*, *BMP4*, and *BMP7* in cetaceans identified signatures of accelerated evolution, with *BMP4* exhibiting lineage-specific amino acid substitutions under positive selection. All detected substitutions were located within the TGF- β propeptide domain, a conserved structural region that facilitates the formation of homodimers, which helps maintains normal biological functions (Robertson et al., 2015). Given that BMPs also form functional heterodimers, which enhance their functional diversity (Hogan, 1996; Pizette et al., 2001; Selever et al., 2004), we speculated that the mutations in cetacean *BMP4* may influence cetacean flipper forelimb formation by affecting BMP homodimer formation. Functional validation

confirmed that cetacean *BMP4* variants and their specific mutations significantly disrupted apoptosis and proliferation, accompanied by altered expression of key regulators in the apoptotic signaling cascade at both transcriptional and protein levels (Figure 5). As limb morphogenesis depends on tightly orchestrated apoptotic programs, particularly the activation of intrinsic mitochondrial pathways (Zuzarte-Luis & Hurle, 2005), such disruption may influence tissue remodeling outcomes. BMP signaling is facilitated by the specific binding of BMP ligands to receptors (BMPRI/II), which subsequently regulates the expression of key apoptotic factors, including Bcl-2 family proteins (e.g., pro-apoptotic Bax and anti-apoptotic Bcl-2) and apoptosis factor Cyt c, to determine tissue fate and ultimately direct cell proliferation, differentiation, and apoptosis (Arakawa et al., 2017; D'orsi et al., 2017; Lindsten et al., 2000; Montero & Hurlé, 2010; Shubin et al., 1997; Tang et al., 2019). Moreover, the morphogenesis of webbed or flipper-like limbs

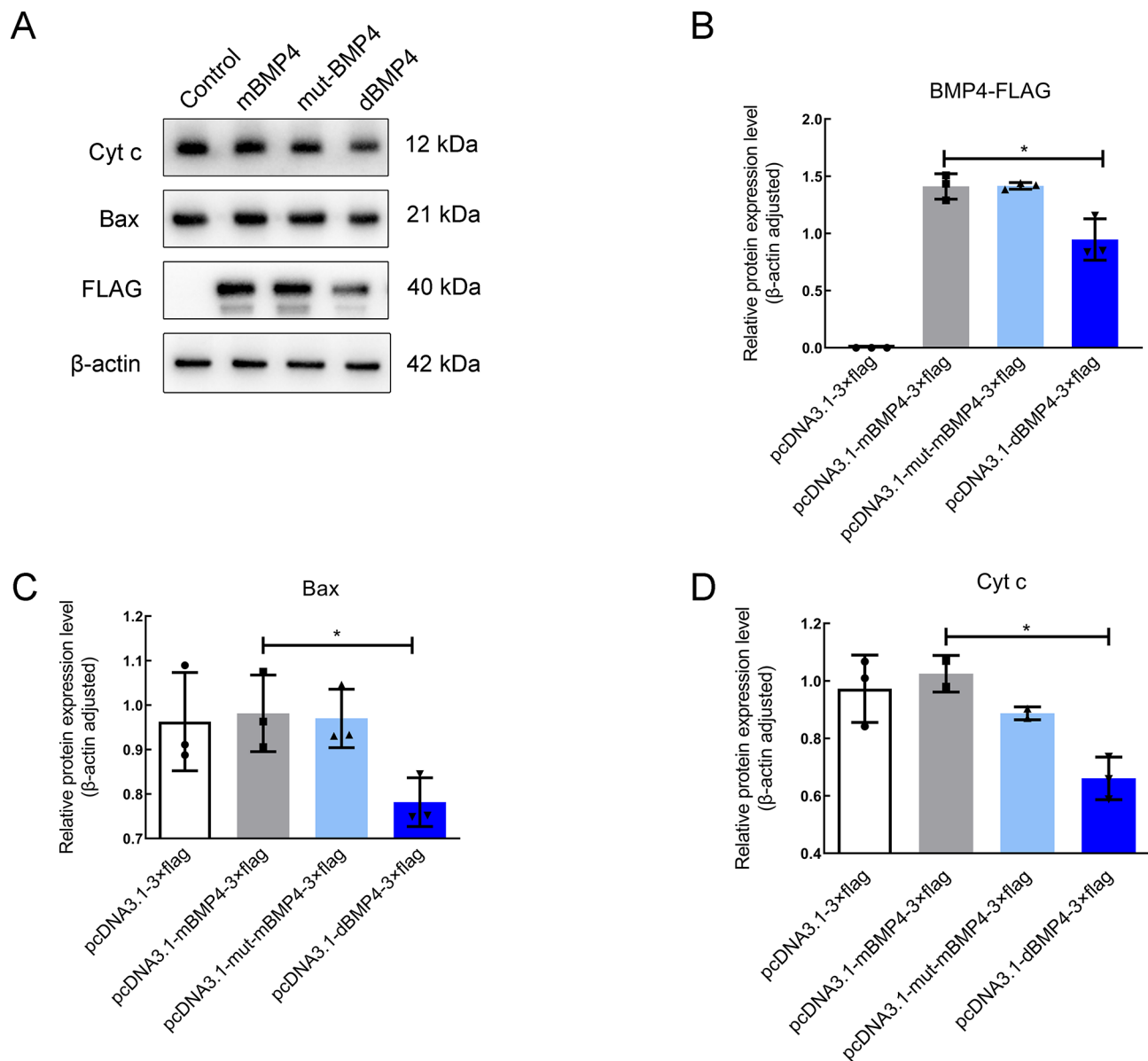


Figure 4 Protein expression of BMP4 and apoptosis-related proteins in transfected NIH3T3 cells

A: Western blot analysis of BMP4 and apoptosis-related proteins (Bax and Cyt c) in NIH3T3 cells transfected with mBMP4, mut-mBMP4, or dBMP4 plasmids. Cells expressing dBMP4 exhibited significantly reduced levels of pro-apoptotic proteins. B–D: Quantification of BMP4, Bax, and Cyt c protein expression. Data are presented as mean±SD, *: $P < 0.05$.

in mammals is frequently attributed to the suppression of apoptosis during embryogenesis (Gavazzi et al., 2023; Weatherbee et al., 2006; Yokouchi et al., 1996). The observed inhibition of apoptotic signaling by cetacean BMP4 variants aligns with this developmental model, supporting the hypothesis that these mutations contribute to the evolutionary retention of interdigital tissue and the emergence of flipper morphologies in cetaceans.

In conclusion, this study provides evidence that adaptive modifications in *BMP4* may underlie a key evolutionary innovation in cetaceans—the transformation of forelimbs into flippers. These findings offer a mechanistic link between molecular evolution and morphological adaptation, highlighting *BMP4* as a central player in cetacean limb specialization. Future investigations integrating regulatory genomics, developmental transcriptomics, and functional assays *in vivo* will be essential for further dissecting the complex genetic architecture that enabled the emergence of cetacean aquatic

limb phenotypes.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

S.X.X. and G.Y. conceived the study and designed the experiments. L.Y.D. collected and analyzed the data. Y.L. wrote the manuscript. J.L. participated in data analysis. S.X.X. and G.Y. reviewed the manuscript. All authors read and approved the final version of the manuscript.

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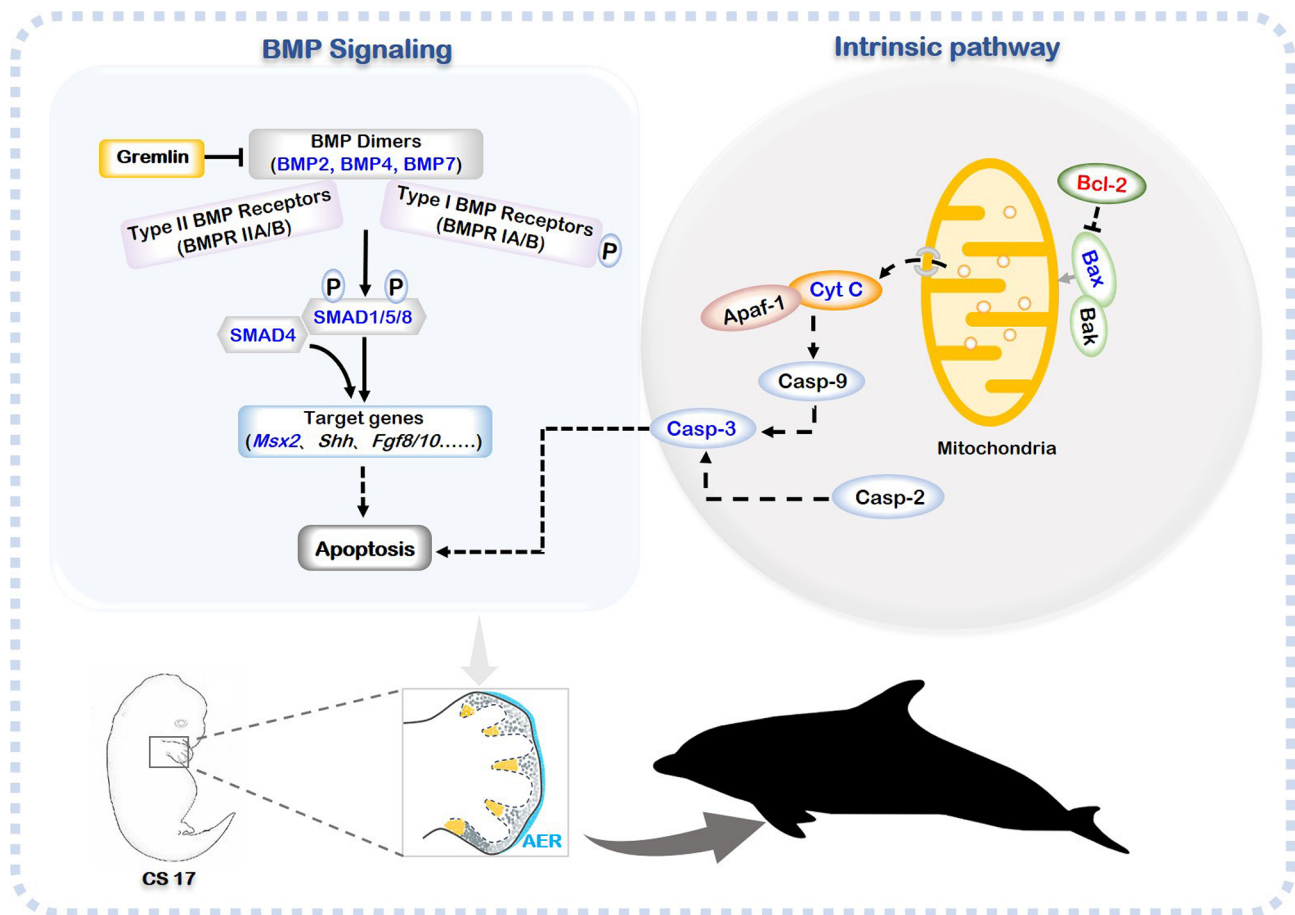


Figure 5 Schematic representation of BMP signaling and intrinsic apoptotic pathways

Left: Schematic of BMP signaling pathway during cetacean limb bud development, incorporating cetacean embryo adapted from Gavazzi et al. (2023). Right: Schematic of intrinsic apoptotic pathway based on Tang et al. (2019). CS17: Carnegie stage 17; AER: Apical ectodermal ridge. Blue and red labels are candidate factors from this study, and arrows represent regulatory relationships.

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