## cDNA Cloning and Expression Analysis of *Mest* Gene in the *Bufo gargarizans*

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**Abstract:** The *Mest* (mesoderm-specific transcript) gene has been considered an imprinting gene in human and mouse, and was also confirmed in other mammals and flowering plants. To investigate the function and evolution of this gene, the cDNA of full length *Mest* gene was obtained using 5'- and 3'-RACE from the Chinese Large Toad (*Bufo gargarizans*). The transcript is 1325 bp in length which contains a complete open reading frame (ORF) encoding a polypeptide of 326 amino acids (GenBank accession number: ABQ10905). There is a typical  $\alpha/\beta$  hydrolase fold domain in the putative gene product, and it shows high similarity to sequence of homologous protein of Xenopus tropicali (86%), mammlian (70% – 80%). RT-PCR (reverse transcriptase-polymerase chain reaction) analysis demonstrated that the *Bufo gargarizans Mest* (*BgMest*) gene is expressed widely in testis, ovary, liver, kidney, spleen, brain, stomach and lung. The conservation of the *BgMest* gene, suggested that the function of *BgMest* was conserved in amphibians. However, the phylogenetic tree of the imprinting gene of the mammals and other vertebrates examined in this study indicated their divergent origins.

Key words: Bufo gargarizans; Mest gene; Imprinting gene; RACE; RT-PCR

## 中华大蟾蜍 Mest 基因的 cDNA 克隆和表达分析

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**摘要:** Mest 基因是一种印记基因,在人、小鼠以及其他的哺乳动物和有花植物中都有研究报道。为了更好地 研究该基因的功能和进化特点,利用 RACE 法获得了中华大蟾蜍 Mest 基因(BgMest)的 cDNA 全长序列(1325bp), 它包含一个完整的 ORF,可编码 326 个氨基酸的多肽(GenBank 登陆号: ABQ10905)。多肽链中包含一个典型的 α/β 水解酶折叠结构域,其在氨基酸水平上与热带爪蟾和一些哺乳动物分别存在 86%和 70%~80%的相似性。进化 树分析显示 Mest 基因为单系起源。RT-PCR 显示, BgMest 基因在精巢、卵巢、肝、肾、脑、胃和肺中都有表达, 并且该基因在序列、表达模式以及蛋白产物的高级结构的高度保守性都说明它在两栖类生物中是保守的。但是在 对哺乳动物以及一些脊椎动物的印记基因进行进化分析时,发现它们具有不同的起源。

关键词:中华大蟾蜍; *Mest* 基因;印记基因; RACE; RT-PCR 中图分类号: Q959.53; Q951.3; Q349; Q78 文献标识码: A 文章编号: 0254-5853-(2009)04-0369-08

Genomic imprinting refers to the parent-of-origin specific gene modification resulting in monoallelic expression of a gene dependent on its parental origin (Brannan & Bartolomei, 1999). The molecular mechanism of genomic imprinting appears to share aspects of epigenetic regulation with X inactivation in human and mouse, since both phenomena involve DNA methylation and asynchronous replication of DNA (Saitoh & Wada, 2000). This phenomenon has been also observed in mammals and flowering plants (Scott &

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Spielman,2004). To date, there are approximately 100–200 imprinting gens in the mammal genome, such as H19, IGF2, IGF2R, XIST, etc....(Looijenga et al, 1997). These special genes in mammals are imprinted so that only one of the parental alleles is actually expressed in target cells. *Mest* (mesoderm-specific transcript)/*Peg1* (paternally expressed gene 1) is an important maternally imprinted mammalian gene which is predominantly expressed in the mesoderm and its derivatives (Reule et al, 1998; Isles et al, 2006). Only the paternal copy of the gene is expressed in interspecific hybrids. This gene has been assigned to 7q32 in human and mapped to chromosome 6 in mouse (Lefebvre et al, 1997; Kosaki et al,2000)

The *Mest* orthologs were studied mainly in mammals, but there were also reports in non-mammalian species like pufferfish (*Fugu rubripes*), zebrafish, *Xenopus tropicalis* and tammar wallaby (Suzuki et al, 2005). The widespread *Bufo gargarizans* is a representative of Bufonidae, Anura, Amphbia, and it plays an important role in the ecological balance of nature. The amphibians represent a bridge in the evolution of vertebrates from aquatic to terrestrial. They have highly specialized morphological and functional characteristics to adapt to different environments. In this paper, we isolated and analyzed the expression pattern of *BgMest*. To understand the function and evolution of this gene, the phylogenic tree was also constructed.

#### **1** Materials and Methods

#### 1.1 Animals

Two male and two female adult *B. gargarizans* were obtained from the suburb of Wuhu city, Anhui province, China. Tissue from the testis, ovary, liver, kidney, spleen, brain, stomach and lung were dissected from the toads. Samples were snap frozen in liquid nitrogen, and stored at  $-80^{\circ}$ C until use.

#### 1.2 Cloning of Mest cDNA

To amplify the conservative fragment of *BgMest* cDNA (424bp), a pair of primers (M1: GATTGCCCT-GGACTTTAT, M2: TACACCTGTCGGGAGTCC) were designed according to the conserved amino acid sequences that have been found in human (Accession number: NP\_803490), and mouse (Accession number: AAM78507). To obtain the complete *BgMest* cDNA, rapid amplification of cDNA ends (RACE) technique was performed (Invitrogen, Tokyo, Japan) according to instructions.

Total RNA was extracted from toad testis with

TRIzol (Invitrogen) according to the manufacturer provided protocol. Single-stranded cDNA was prepared with SuperScript TM III reverse transcriptase, following the operating instruction. For the 3'-RACE, M1 and anchor primers (5'-CGTCTAGAGGTACCGGATCCA-ACA-3') were used. The PCR reaction consisted of 30 cycles of 95°C (30 s), 65°C (30 s) and 72°C (3 min), ending with a 7min extension step at 72°C. After cDNA fragments had been obtained by 3'-RACE, they were inserted into the PCR2.1 vector and sequenced. For the 5'-RACE, single-stranded (ss) cDNA was reverse-transcribed from total RNA according to the manufacturer's protocol (Invitrogen, Tokyo, Japan). Oligo dA was added to the ss cDNA by terminate deoxynucleotidyl transferase according to the protocol (TOYOBO, Tokyo, Japan), which used as a template for the PCR. The PCR was performed by using M2 and oligo dT anchor (5'-CGTCTAGAGGTACCGGATC-CAACAT(17)V-3') primers for 30 cycles under the following conditions: denaturation at  $95^{\circ}$ C for 30 s, annealing at 65°C for 30s, extension at 72°C for 2min, and ending with an extension step at  $72^{\circ}$ C for 7 min. After cDNA fragments had been obtained by this procedure, they were inserted into the PCR2.1 vector and sequenced.

# **1.3 RT-PCR analysis and tissue expression of** *Mest* gene

To analyze the tissue specificity of *Mest* gene expression, total RNA was extracted from several tissues including testis, ovary, liver, kidney, spleen, brain, stomach, lung of male and female specimens. It was prepared for homogenizing the tissue in 100 uL Trizol reagent (Takara, China) and 50 uL chloroform. The aqueous phase was precipitated in 150 uL isopropanol at  $4^{\circ}$ C overnight. The RNA pellet was rinsed in 75% ethanol and resuspended in 12 uL DEPC-treated ddH<sub>2</sub>O and immediately used for RT-PCR. Reverse transcriptase polymerase chain reaction (RT-PCR) amplifications were performed using two degenerated primers (M1 and M2) The nucleotide sequences of the primers used were EFI $\alpha$ , H1: TCCACCACCACCAGCAACAAT.

RT-PCR was carried out using 3uL total RNA per reaction,  $1 \times$  buffer reaction mix and  $0.2 \mu$ mol/L b-actin primers or  $0.3 \mu$ mol/L of *Mest* primers in separate tubes, in a total volume of 20  $\mu$ L. All reactions for *Mest* contained 6% DMSO. cDNA synthesis was performed at 50°C for 30 min and amplification conditions were: 94°C /5min for initial denaturation; 94°C/30s for denaturation,

 $56 \degree C/60 \text{ s}$  for annealing,  $72 \degree C/60 \text{ s}$  for extension, 35 cycles;  $72 \degree C/10 \text{ min}$  for final extension. Each PCR product was detected by electrophoresis on 1.2% agarose gel.

#### 1.4 DNA sequencing and bioinformatical analysis

Homology analysis of the amino acid sequence alignments of *BgMest* and other vertebrates including *Homo species* (Accession number: NP\_803490), *Mus musculus* (Accession number: AAM78507), *Macaca mulatta* (Accession number: XP\_001098320), *Gallus gallus* (Accession number: XP\_425258), *Xenopus tropicalis* (Accession number: NP\_001005789), *Takifugu rubripes* (CAB96533), and *Danio rerio* (Accession number: CAK04921) was performed with Clustal X1.81 software. Phylogenetic tree construction was achieved using MEGA V2.1 (Molecular Evolutionary Genetic Analysis) program of neighbour-joining (NJ) methods. Analysis of protein secondary structure was finished by predictprotein program.

#### 2 Results

#### 2.1 Sequence analysis of *BgMest* gene

To isolate the *BgMest*, the complete cDNA were amplified by 5' and 3'-RACE technique, a 1325 bp *Mest* cDNA encoding a predicted protein of 336 amino acids was obtained. The cDNA sequence included 34 nucleotide acids in 5'-UTR, 310 nucleotide acids in 3'-UTR, and a complete open reading frame (ORF) which conformed to several characteristics that GC content of cDNA sequence is high but the sequence is short in 5'-UTR. Fig.1 showed the full-length *Mest* cDNA of *B. gargarizans*. This *BgMest* sequence was determined by comparison with orthologous proteins of the amino acid sequences using BLAST (Accession number is ABQ10905 in the NCBI database).

The putative amino acid sequences of *BgMest* were aligned with orthologous proteins from *Homo species*, *Mus musculus*, *Macaca mulatta*, *Gallus gallus*, *Xenopus tropicalis*, *Takifugu rubripes* and *Danio rerio*. The *BgMest* showed a significant sequence homology with those of other vertebrate *Mest* proteins. It exhibited the highest similarity to *Xenopus tropicalis Mest* (86% identities) and the *BgMest* is similar to other vertebrates with 70% – 80%nt identities (Fig.2).

Additionally, based on amino acid sequence of the *Mest* protein, they contained a conserved domain named  $\alpha/\beta$ -hydrolase fold domain. It conformed to the 3-D model of the *Mest* protein (Fig. 3). According to analysis via Phyre program, the model belonged to the

 $\alpha/\beta$ -hydrolase family. Fig. 2 showed that this domain was located at amino position 88 – 320 in mammal, at 105 – 337 in zerbrafish and at 64 – 296 in *Gallus gallus*. The domain of *BgMest* protein was located at position 107 – 321. This conserved domain was determined to be prominently expressed in mesodermal derivatives during embryogenesis only from the paternally inherited allele, and was detected in a number of proteins of several animals, plants and microorganism (Kamei et al, 2007; Yun et al, 2002).

Analysis of protein secondary structure (Fig. 4) and 3-D model of *BgMest* protein can understand its function and recognize the interactions within proteins, which is important in biological and medical research. Protein secondary structure of *BgMest* gene is composed of helix (h), strand (s) and coil (c) like the *Mest* gene of mammals. It includes six helixes, six strands and sixteen coils within the *BgMest* protein (Fig. 4). It was shown that the *BgMest* protein also contains a conserved  $\alpha/\beta$ -hydrolase fold domain by analysis 3-D model of *BgMest* protein (Fig. 3).

#### 2.2 Phylogenetic tree of Mest gene

The relationships between the proteins encoded by BgMest gene and the corresponding proteins from other species were analyzed using neighbour-joining (NJ) method (Fig. 5). The tree showed that all mammalian proteins cluster together and the fish and amphibian cluster into another branch. This may suggest the divergent origins separating mammalians from the other vertebrates including teleosts, avian and amphibians. *Mycobacterium tuberculosis C* belonged to an actinomyces, which was separated from the vertebrate. The results were identical with traditional classification, which supports the *Mest* protein monophyly.

#### 2.3 Expression of BgMest

To determine the multiple tissue expression of this gene, RT-PCR was carried out on various tissues (including testis, ovary, liver, kidney, spleen, brain, stomach and lung) in adult *B. gargarizans*. As a control, cytoplasmic EFI-*a* gene from *B. gargarizans* was used. As seen in Fig. 6, all the aforementioned tissues were expressed.

#### **3** Discussion

The genomic structure of *Mest* gene has been shown to include a  $C_PG$  island in human, mouse and zerbrafish (Reule et al, 1998; Kosaki et al, 2000; Hahn et al, 2005). A characteristic of imprinted genes is that maternal and paternal alleles show differences in methylation position

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I GG G TG W AG S GG GA D CC P	F S TATCG I C GGATA D F TATTT I L AGCTTI A L TCCAG P V CAAGTI K S	P GTGA TGTG TGTG TGAC TGAC TGAC TCAA CAAC	AATA I GGGT GGAC T GTA GTA V CAAA N TCCC P CCTT F	ACTA L F AGC A TATA I TATA S CCA H TAC T	TGG G CCT L AAAA N ATCO S TCC P TGT.	AGA R ACC P CAG R CCA Q GGT V TGA E ACTO L	ACTO L TTA GGAT I GCG R TCC/ P ATTO JGA D	JATC M TAC" T CAA N CAG R ATTC L CTTC L D GAT	JAAG N CGA D AAA K JCA H JGA D ICAG H	CTTT F GCCT F GTGG GTA GTA F TTTA L F CATC I	TAC Y FTC/ S CA/ N CAC R ATI I ATA V CAG S	TTC F AGA D ATC L JAG, E TTAJ Y CAA K TCA H	TTT F CAC T TAG TAG AAO R G G AAO G G AA K CTA Y	AGC S TGA FAG GCT Q ACC P ACC L CCC P	AAAA K ATAC Y TAGAC J GGGTC J V ACTG L TATT I TCAG Q	652 703 : 754 } 805 856 907 958
I GG G TG TG W AG S GG GA D CC C C T	F S TATCG I C GGATA D F TATTT I L AGCTTI A L TCCAG P N CAAGT K S GGAGG	P GTGA TGTGG TGTGG TGTGTG TGACA TGACA TGACCO T GACCO	AATA I GGGT V GGAC T GTA GTA V CAA P CCTT F CAA	ACT I CATT F CAGC A TATC S CCA H TAC T TGG	TGG G CCT L AAAA N ATC S TCC P TGT V ATT	ragi R ACC P CAG R CCA Q GGT V TGA E ACTC L CCT	ACTO L TTA GGAT I GCG R TCC/ P ATTO F GGA GAA	JATC M TAC' T CAA N CAG R ATTC L CTTC L D GA' D TGC	JAAA N CCAA Q CCGA D AAAA K JCAA H JCAA D CCAA H TTA	ETTI F GCCI F ATGG G ATGG G ATGG V FTTA L FCAA Q CATC I TCTA	TAC Y TTC: S CAI N CAC R AATI I AATA Y CAG S AAAA	TTC F AGA D ATC L JAG, E TTAT Y CAA K TCA H CTT	TTTT F CAC T TAG <sup>7</sup> V AAO R CGGA K CTA X TAT	AGC S TGA E TAG TAG GCT V ACC L CCC P CAA	AAAA K ATAC Y TAGAC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCA	652 703 754 805 856 907 958 1009
I GG G TG W AG S GG GA D CC C C L	F S TATCG I C GGATA D P TATTT I L AGCTTI A L TCCAC P N CAAGT K S GGAGC E I	P GTGA TGTGG TGTGG TGTGTG TGACA TGACA TGACCO T GACCO P	AATA I GGGT V GGAC T GTA V V CCAA V V CCAA C CCAC T	ACTA L TATT F CAGC A TATA I TTC. S CCA H TACC T TACC T G G	TGG G CCCT L AAAA N AATCO S TCCC P TGT. V AATTI F	ragi R ACC P CAG CCA Q GGT V TGA E ACTO L CCT L	ACTC I TTA' Y GGAT I GCCG R TCC/ P ATTC F GGA' D GAA N	JATC M TAC' T CAAC N CAG R CAG R CAG R CAG C L CTTC L CTTC L D TGC A	JAAG N CCA Q CCGA D AAAA K J CAA H J GAA D GAA D CAA D CAA D T CAA Y	PTTT F GCCT F GTA GTA V TTTA L CCATO L I TCTA L	TAC Y TTC: S CAI N CAC R AATI I XTAC Y CAG S AAAA N	TTC F AGA D ATC L JAG. E TTAT Y CAA K TCA H CTT F	TTTT F CAC T TAG V AAAO R CAAO R CGGJ G GAA K CTA K TAT I I	AGC S TGA E TAG GCT V GCT V ACC L CCC P CAA N	AAAA K ATAC Y TAGAC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCA S	652 703 754 805 856 907 958 1009
I GG G TG W AG S GG GA D CC C T C T T	F S TATCG I G GGATA D M TATTT I L AGCTT A L TCCAGT K S GGAGG E I IITGAG	P GTGA GTGA TGTGA TGAC TGAC TGAC TCAA CCAA C	AATA I GGGT V GGAC T GTA V CCAA P CCAC T F CCAC T	ACTA L TATT F CAGC A TATA I TTC S CCA H TAC T TGG G G A	TGG G CCTT L AAAA N AATCO S TCCC P TGTA V AATT F CAA	ragi R ACC CAG R CCAG R CCAG Q GGT V TGA E ACTO L CCTV L CCTV L	ACTC L TTA' Y GGAT I GCG R TCC/ P ATTC J GAA N CAAT	JATC M TAC' T CAA N CAA R CAG R CAG R CAG L CTTC L TGC A TTGC	JAAG N ICAG Q CGA D AAAA K JCAG H JGA TCAG H TTA Y GTG	P P C C C C C C C C C C C C C C C C C C	TAC Y TTC: S CAI R CAC R AATI I CAG S AAAA N N	TTC F AGA D ATC L JAG. E TTAI Y CAA K TCA K CTT F CCA	TTTT F CAC T TAG V AAO R CAA C G AA C C TA K C TA T TAT I TTC	AGC S TGA E TAG GCT V ACC P ACC L CCC CAA N	AAA K ATAC Y TAGAC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCA S 3TGA	652 703 754 805 856 907 958 1009
I GG G TG W AG S GG GA CC P CT L TT F	F S TATCG I G GGATA D N TATTT I L AGCTT A L TCCAGT K S GGAGG E I ITGAG	P GTGA GTGA TGTGA TGAC TGAC TGAC TCAA CCAAC TGACCC T GACCCC	AATA I GGGT V GGAC T GTA V CCAA V CCAA V CCAC T T AAAA	ACTA L TATT F CAGC A TATA I TTCC S CCA H TACC T TGG G GAA	TGG G CCT L AAAA N AATC S TCC P TGT. V AATT F CAA	RAGA RACC PCAG RCAG CCAG QCAG QGT V TGA. ECA L CCT L CCT L	ACTC L TTA' GGAT I GGGG R TCC/ P ATTC J GGA D GAA N XAAT	JATC M TAC' T CAA N CAA N CAG R CAG R CAG R CAG L CTTC L CTTC L TGC A TTG	JAAG N FCAG Q CGA D AAAA K JCAA K JCAA H JCAG TCAG TTA Y GTG	PTTT F GCCT P ATGG GTA V CGTA CGTA L CCAF C CATC I TCTA L	TAC Y TTC: S CAC R CAC R AATI I CAC R CAC R S AAA N N ATT	TTC F AGA D ATC L JAG. E TTAI Y CAA K TCA K TCA F TCA	TTTT F CAC T TAG' V AAAO R CAA G GAA K CTA CTA T TTC'	AGC S TGA E TAG GCT V GCT V ACCA P ACCA L CCCC CAA N TGTO	AAAA K ATAC Y TAGAC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCA S 3TGA	652 703 754 805 856 907 958 1009
I GG G TG W AG S GG G G C C C C T C T T F F	F S TATCG I G GGATA D N TATTT I L AGCTT A L TCCAG P N CAAGT K S GGAGG E I I (TGAG	P GTGA GTGA E TGTGA G TGAC T TGAC T CCAA C CCAC	I I GGGT V GGAC T GTA V CAA N CCAA N CCAC F CCAC T KAAA	ACTA L CATT F CAGC A TATA I TTTC. S CCA H TAC T TGG GAA	TGG G CCCT L AAAA N AATCO S TCCC P TGT. V AATT F CAA	ragi R ACC CAG R CCAG Q GGT V TGA. CCA V TGA. E CCA V TGA. E CCT L CCT L	ACTO L TTA' Y GGAT I GGCG R TCC/ P ATTO F JGAA N 'AAT	M TAC' T CAA N CAAG R ATTC L CAG R ATTC L CAG TTC L TTC TTC A TTC	JAAG N ICCAG Q CGA D AAAA K J CAAA B GAA TTA TTA Q TTA	PTTT P GCC1 P GTA GTA CATGG GTA V TTTA L TCTA L TCTA L	TAC Y TTC: S CAC R CAC R CAC R CAC R CAC R CAC R S CAG S S AAAA N N ATT	TTC F AGA D ATC L JAG E TTAI Y CAA K TCA K TCA K TCA	TTTT F CAC T TAG' V AAAO R AAO R CAA K CTA K CTA K TAT I TTC'	AGC S TGA E IAG GCT V ACC P ACC C C C A C C C A C C A C C A C C C A C C C A C	AAAA K ATAC Y TAGAC 7 D GGGTC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCA S 3TGA	652 703 754 805 856 907 958 1009 1060
I GG G TG W AG S GG G G C C C C C T T T F A T	F S TATCG I G GGATA D N TATTT I L AGCTTI A L TCCAG F N CAAGT K S JGAGG E I I (TGAG	GTGA GTGA TGTGA TACA TACA TACA TCAA TCAA	I GGGT V GGAC T GTA V CAA N TCC P CCAC P CCAC T F CCAC T T CAA A CCAC C T CAA C CAA C C C C C C C C C C C C C	ACTA L CATT F CAGC A TATA I TATA TATA TAC TAC G GAA TAA	TGG G CCCT L AAAA N AATCO S TCCC P TGT. V AATT F CAP	ACC P CAG CCAG Q CCAG Q GGT V TGA E ACT( L CCT L CCT L CCTI L AGTI	ACTO L TTA' Y GGAT I GGCG R TOCA P AATTO F GGAA N TAAT AAAC	M TAC' T CAA N CAAG R ATTC L CAG R ATTC L CTTC L IGA TGC A TTG AAG	AAAA N CGA CGA D AAAA K GGA L CAA CAA D CAA D CAA D CAA D CAA D CAA CAA	PTTT P GCC1 G GTA CATGG G GTA V TTTA L TCTA L TCTA L TCTA L	TAC Y TTC/ S CA/ N CAC R ATA Y CAC S AAAA N ATT TAA	TTC F AGA D ATC' JAG E TTAI Y CAA K TCA K TCA F TCA	TITT F CAC T TAG' TAG' V V V V V CAC TAG' GAA CAA CAA CAA CAA CAC CAC CAC	AGC S TGA E IAG GCT V ACC L CCC P CAA C CAA N IGTC	AAAA k ATAC Y TAGAC 7 D GGGTC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCA S 3TGA CACA	652 703 754 805 856 907 958 1009 1060
I GG G TG W AG G G G G G C C C C T T T F A T	F S TATCG I G GGATA D N TATTT I L AGCTI A L TCCAG F S GGAGG E I I TCAG F I I TCAG F I CAAGT K S GGGCI ATGGA	P GTGA TGTGA TGTGA TGTGA TGAC TGAC TCAA CAAC CAAC	AATA I GGGT V GGAC T GTA V CCAA V CCAA V CCAA V CCAA T CCAA T C T T AAAA	ACTA L ATT F CAGO A TATT TTTC. S COCA H TAC TAGG G GAA TAA TGG	I TGG G GCCT L AAAA N AATC S TCC P TGT. V AATT F GCAP AGC CTC	ACC P CAG CCAG Q CCAG Q GGT V TGA. E ACT( L CCTI L CCTI L GGTI AAT 3GA(	ACTO L TTA' Y GAT I GCG R TCC/ P AATTO F JGA' N GAA N CAAT	JATC M TAC' T CAA N CAAG R CAAG C CTTC L CTTC L CTTC C TTC C AAAG	JAAG N FCAG Q CGA D AAAA K JCAA H JCAG H TTA GTG GTG	P P SCCI P C G G G G G G G G G C A C C A C C C C C	TAC Y TTC/ S CA/ N CAC R AAA N AAA N AAA N AAATT TAA	TTC F AGA D ATC L JAG TTAI Y CAA K TCA K TCA H CTT F TCA	TITT F CAC T TAG' TAG' TAG' GAA CGGJ GAA K CTA TAT' I TTC' TTTC'	AGC S TGA E IAG GCT V ACC P ACC L CCC P CCA N N IGTC	AAAA k ATAC Y TAGAC 7 D GGGTC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCA S STGA .CACA GCCAG	652 703 754 805 856 907 958 1009 1060
I GG G TG- W AG G G G G C C C C T C T T F A T A T	F S TATCG I G GGATA D N TATTT I L AGCTI A L TCCAG P N CAAGT K S GGAGG E I I TGAG ATGGA GGGCI ATGGA	P GTGA TGTGA TGTGA TGTGA TGAC TGAC TCAA CAACCO P CCAAC CAACCO TCAAC CCACCO TCAAC	I GGGT V GGAC T GTA V CCAA V CCAA V CCAA V CCAA T CCAA T CCAA T T GGA T GGA	ACTA L ATT F CAGO A TATA TTTC. S COCA H TAC G G A TAC TAC TAC TAC TAC TAC TAC	AACT T TGG G CCCT L AAAA N AATC S TCCC P TGT. F AATC F CAA AGC TCTC TCTC	raga R P CAG R CCAG R CCAG Q GGT V TGA E ACT(C L L CCTT L AGTI AGTI AGTI AGTA	ACTO L TTA' Y GAT I GCG R TCC/ P ATTO P GAA N CAAT CAAT	JATC M TAC' T CAA N CAAG R CAAG R CAAG C TTC C TTC C TTC C AAAG C TCC	JAAG N FCAG Q CGA D AAAA K JCAA K JCAA H JCAA H TTA TTA QTG QTG	P P SCC1 P G G G G G G G G G G G G G G G C A C C C C	TAC Y TTC: S CAD R CAC R AAAA Y CAG S AAAA N ATT TAA CAG CCT	TTC F AGA D ATC' L JAG E TTAI Y CAA K TCA K TCA H CTT F F CA	TITT F CAC T TAG' V V AAAO R R GGAA CTA CTA TTATT I TTC' TTTT ACCC ATA	AGC S TGA E IAG GCT V ACC P ACC L CCC P CAA N IGTC CCA N TGTC	AAAA K ATAC Y TAGAC Y GGGTC Y C GGGTC Y C ACTG L TATT I TCAG Q TTCAG S TTCA S GGA ACTG L CACA S CACA CACA CACA	652 703 754 805 856 907 958 1009 1060
I GG G TG W AG S GG GA CC P CT L TT F AT AC	F S TATCG I G GGATA D P TATTT I L AGCTT A L TCCAG P Y CAAGT K S GGAGG E I I TGAG ATGGA GGGCT TCACT	GTGA GTGA TGTGA TGTGA TGAC TGAC TGAC TCAA CAACC TCAAC CCAC CC	AATA I GGA GGAC T GTA V CCAA N CCAA N CCAA N CCAA T CCAA T CCAA T CCAA T CCAA T CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAAA CCAAAA CCAAA CCAAAA CCAAAA CCAAAA CCAAAAA CCAAAA CCAAAAA CCAAAAA CCAAAAAAAA	ACTA L TATT F CAGC A TATA I TTC S CCA H TAC T TGG G GAA TAA TGC TAG TAG	AACT T TGG G CCT L AAAA N AATO S TCC' P TGT. V CAP AGC TCT TCT TCT TCT	TAGA R ACC P CAG R CCA Q GGT V TGA E ACTC L CCT L CCT L CCT L CCT L CCT L CCT L CCT L CCT L CCT L CCT L CCT L CGA C ACC Q Q Q Q C ACC Q Q Q Q Q Q Q Q Q	ACTO L TTA' Y GAT I GCG R TCC/ P ATTO P GAA N TCC/ D GAA N 'AAT CTA/ ACA' CCT	JATC M TAC' T CAA N CAAG R CAAG R CTTC L C'TTC L C'TTC C TTC C C'TTC C AAAA C'TC C'AAG C'TC C'CC C'CC C'CC C'CC C'CC C'CC C'C	JAAG N ICAG Q CGA D AAAA K JCAG H GGA GTG H TTA Q GTG GTG ICAG C ACC ICAG C ICAG C ACAC ICAG ICAG	P P SCC1 P G G G G G G G G G G G G G G G C A G C A G G C A G G C A G G C A G G C A G G C A C C C C	TAC Y TTC: S CAD N CAC R ATT I CAG S AAAA N ATT TAA CAG CCT: ATT	CTTC F AGA D ATC' L JAG E TTAI Y CAA E TTAI Y CAA H CTT F F CA A CA GGI	TITT F CAC T TAG' V V AAAO R R GGAA K CTA CTA TTC' TTTT ACCC ATA AGAA	AGC S TGA E IAG GCT V ACCA P ACCA P ACCA C ACT C CAA N IGTC CCAA CTAA CTAA	AAAA K ATAC Y TAGAC 7 D GGGTC 7 V ACTG L TATT I TCAG Q TTCAG S GTGA SCACA SCCAG TTTTA IGCAT	652 703 754 805 856 907 958 1009 1060
I GG G TG W AG S GG GA D CC P CT L TT F AT AC AC GT	F S TATCG I G GGATA D F TATTT I L AGCTI A L TCCAG P Y CAAGT K S GGAGG E I I [TGAG GGGCI ATGGA GGGCI TCACT TCCGA	GTGA GTGA TGTGG W TACA Q TGAC TGAC TCAA CAAC CAAC CAAC CAAC CAAC	AATA I GGA GGAC T GTA V CCAA V CCAA V CCAA V CCAA V CCAA V CCAA V CCAA V CCAA V CCAA V CCAA CCCC CCAAA CCAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAA CCAAAA CCAAAA CCAAA CCAAAA CCAAAAA CCAAAA CCAAAAA CCAAAA CCAAAAA CCAAAAA CCAAAAAAA CCAAAAAAAA	ACTA L TATT F CAGC A TATA I TTC S CCA H TAC TAC G GAA TAA TGC GAA TAA TGC CAG	AACT T TGG G CCT L AAAA N AATO S TCC' P TGT. V F AATT F CAA AGC TCT TCT TCT TCT	TAGA R ACC P CAG R CCAG Q GGT V TGA. E ACT( L CCTI L CCTI L GGTI L GGTI L AAT AAT AAT AAT AAT AGTI TTA.	ACTIC L TTA' Y GAT I GCG R TCC/ P GAA N GAA N AATIC P GAA N AATIC CTA/ AAA CCTA/ AAA	JATC M TAC' T CAA N CAG R ATTC L CTTC L CTTC L TGC AAG AAA CTG' GGC AGC.	JAAG N ICAG Q CGA D AAAA K JCAG A H GGA GTG GTG GTG GTG GTG GTC AAA	P P G G G G G G G G G G G G G G G G G G	TAC Y TTC: S CAC N CAC R ATT: CAC CAC S AAAA N TAA CAC CAC CAC CAC CAC CAC CAC	TTIC F AGA D L SAGA TCA E TCA E TCA E TCA A CAT F CA A CA A CA A CA A CA A	TITT F CAC T TAG' TAG' G GAA CTA G GAA K CTA TATC I TTC' I TTC' CCC AAAO	AGC S TGA E FAG GCT V ACC P ACC L CCC P CAA CCC N CCAA N GCT CCAA N CCAA CCAA CCAA CCAA CCAA CCAA	AAAA K ATAC Y TAGAC GGGTC V V ACTG L TATT I TCAG Q TTCAG S GTGA S GTGA ACACA S CACA S GTGA	652 703 754 805 856 907 958 1009 1060

Fig. 1 *Mest* gene nucleotide and deduced amino acid sequence of *Bufo gargarizans* Nucleotides are numbered up and deduced amino acids are below. ATG: initiation codon; TAG: termination codon.

(Georgiades et al, 2001; Edwards et al, 2007). In the researches of huamn and mouse, imprinted role of *Mest* gene was related to the methylation of  $C_PG$  island (Reule et al, 1998; Kosaki et al, 2000). However, unlike the  $C_PG$  island of normal and biallelically expressed genes, the  $C_PG$  island of *Mest* is exclusively unmethylated on the expressed paternal allele, but fully methylated on the silenced maternal allele (Reule et al, 1998). In human

and mouse, *Mest* gene was expressed in amnion, brain, heart, lung, stomach, kidney and liver. Mammalian *Mest* gene is widely expressed throughout the embryo, mostly in mesodermal tissues (Reule et al, 1998; Kosaki et al, 2000). In our study, the *Mest* gene was also expressed in testis, ovary, liver, kidney, spleen, brain, stomach and lung of the adult *B. gargarizans*. And there is high similarity of the protein sequences and evolutionary

Xenopus	MKEWNIQVGLLTVPFLAVYLHIPPPNFSPALFTWRSTGAFFTF
Bufo	MKEWNIQVGLITVPLLAVYLHIPPPNLSPALFTWRSFGAFFTF
Gallus	MKEWWVQVGLLSVPLLAVYLHIPPPRLSPALLSWKASGG
Macaca	MREW#VQVGLLAVPLLAAYLHIPPPQLSPALHSWKSSGKFFTY
Homo	MREW#VQVGLLAVPLLAAYLHIPPPQLSPALHSWKSSGKFFTY
Mus	MREW#VQVGLLAVPLLAAYLHIPPPQLSPALHSWKTSGKFFTY
Danio	${\tt MSGDSPNDSECGSARRVSREWWLHVGLLCVPLLAVYLHIPPPQLSPALNSWRTSGHFFTF}$
Taki fugu	MREWWLHVGLICIPLVAVYLHIPPPQLSPALQKWQSAGDVFHF
	:***::***: :*::*:*********::*******::*****
Xenopus	$KEQQIFYRDS {\tt W} GAVGSSDVVILL {\tt H} GFPTSSYD {\tt W} YKI {\tt W} EGLTQRF {\tt H} RVIALDFIGFGFSDK$
Bufo	$\label{eq:constraint} QEQHIFYRES \verb"WGAVGSSDVVIILHGFPTSSYD""YKI \verb"WEGLTQRFQRVIALDFIGFGFSDK" \\$
Gallus	NWGLRGRYLGPKKSLLFLGLTQRFHRVIALDFVGFGFSDK
Macaca	KGLRIFYQDSVGVVGSPEIVVLLHGFPTSSYDWYKIWEGLTLRFHRVIALDFLGFGFSDK
Homo	KGLRIFYQDSVGVVGSPEIVVLLHGFPTSSYDWYKIWEGLTLRFHRVTALDFLGFGFSDK
Mus	KGLRIFYQDSVGVVGSPEIVVLLHGFPTSSYDWYKIWEGLTLRFHRVIALDFLGFGFSDK
Danio	${\tt RGNDIFY} KESVGVVGSSDVLVLL {\tt HGFPTSSYDWYKI {\tt WDSLTQRFNRVIALDFLGFGFSDK}$
Taki fugu	${\tt RGNKIFYRDSYGALGSSDVLILLHGFPTSSYDWHKIWEPLALRFHRVIALDFLGFGFSDK}$
	. * * * * ** :: *: **:***************
Xenopus	PRLHRYSTFEQASIVEAMIGHLGLRDQKVNLLSHDYGDTVAQELLHRYNHRRKGYINIGS
Bufo	PRFHRYSIFEQASIVEALISHLGLTDQKVNLLSHDYGDTVAQELLYRYEHQRQGHINIGS
Gallus	PRPHHYSIFEQASIVERLVRHLGLHHQRINLLSHDYGDTVAQELLHRYEHNKTGSILINS
Macaca	PRPHHYSIFEQASIVEALLRHLGLQNRRINLLSHDYGDTVAQELLYRYKQNRSGRLTIKS
Homo	PRPHHYSIFEQASIVEALLRHLGLQNRRINLLSHDYGDIVAQELLYRYKQNRSGRLTIKS
Mus	$\label{eq:predistregasive} PRPHQYSIFEQASIVESLLRHLGLQNRRINLLSHDYGDIVAQELLYRYKQNRSGRLTINS$
Danio	eq:prphrysifeqasvvealvahlglseqrinilshdygdtvalellyrsdhnrsghiivns
Taki fugu	eq:prp-hkysifeqasvvealvahlglsnqrvnlishdygdtvalellyrsdqnrtghltlns
	** *:*******:** :: **** .:::*::****** ** ***:* .:.: * : : *
Xenopus	LCLSNGGIFPETHYPRFIQKLLKDGGIFSPILTRIMNFYFFTKGISEVFGPHTQPSEAEY
Bufo	LCLSNGGIFPETHHPRLIQKLLKDGGIFSPILTRIMNFYFFSKGIGEVFGPYTQPSDTEY
Gallus	LCLSNGGIFPETYYPRFIQKVLKDGGLLSPIITRIMNFFFFSRGLGAVFGPYTQPSQAEY
Macaca	LCLSNGGIFPETHRPLLLQKLLKDGGVLSPILTRIMNFFVFSRGLTPVFGPYTRPSESEL
Homo	LCLSNGGIFPETHRPLLLQKLLKDGGVLSPILTRIMNFFVFSRGLTPVFGPYTRPSESEL
Mus	${\tt lclsnggippethrplllqkllkdggvlspiltrimnffvfsrgltpvfgpytrptesel}$
Danio	${\tt LCLSNGGIFPETHHPRFLQKVLKDSGFISPVLTRLMNFQLFSRGIKEVFGPYTQPTEAEV}$
Taki fugu	${\tt LCLSNGGLFPETHHPRLLQTLLKDSSFLAPLLTRLTNFMIFQKGIGEVFGPYTQPTNADF}$
	***************************************
Xenopus	$\verb wdmwtalrtnegnlvidsvlqfinqrskhrdrwvgalinttvplhliygpldpvnphpef  $
Bufo	$\verb wdmwtalrindgnlvvdsilqyinqrrkyrerwvgaltnssvplhliygpldpvnphpef  $
Gallus	$\label{eq:construction} \verb  wdmwtavrtndgnlvvdsilqyinqrkkhrdrwvgalmstsvplhliygpldpvnphpef $
Macaca	$\label{eq:constraint} wdmwagirnndgnlvidsllqvinqrkkfrrrwvgalasvtipihfiygpldpvnpvpef$
Homo	$\label{eq:constraint} \verb"WDMWAGIRNNDGNLVIDSLLQYINQRKKFRRRWVGALASVTIPIHFIYGPLDPVNPYPEF$
Mus	WDMWAVIRNNDGNLVIDSLLQYINQRKKFRRRWVGALASVSIPIHFIYGPLDPINPYPEF
Danio	WDMWTGIRFNDGNLVMDSLLQYINQRLKHRERWVGALTSTLTPLHMIYGPLDPVNPHPQF
Taki fugu	$\label{eq:constraint} wdmwssirvndgnlvmdsilqvinqrlkhrerwvgaltstfvplhmivgpldpvnphpqf$
	****: :* *:****:**:**:**:***** *:* ******
Xenopus	IEHYKNMIPKSTFSILDDHISHYPQLEDPTGFLNAYLNFINSF
Bufo	LDQYKKLIPKSTFTVLDDHISHYPQLEDPTGFLNAYLNFINSF
Gallus	LQLYKKVLPMSTVSVLDDHISHYPQLEDPTGFLNAYLNFINSF
Macaca	LELYRKTLPRSTVSILDDHISHYPQLEDPMGFLNAYMGFINSF
Homo	LELYRKTLPRSTVSILDDHISHYPQLEDPMGFLNAYMGFINSF
Mus	LELYRKTLPRSTVSILDDHISHYPQLEDPMGFLNAYMGFINSF
Danio	LQLYQKLYQRSTVSVLDEHVSHYPQLEDPTGFFNAYLSFINSF
Taki fugu	IRLYQQLVQRSTLTILDEHISHYPQLEDPTGFLNAYFNFIHSF
	: *:::: **.::**:*:*********************

Fig. 2 *Mest* amino acid alignment of the *Bufo gargarizans* (GenBank accession number: ABQ10905) The identical amino acid residues are indicated (\*), the  $\alpha/\beta$ -hydrolase fold domain was boxed by straight line. Accession numbers of other vertebrates are *Homo species* (NP\_803490), *Mus musculus* (AAM78507), *Macaca mulatta* (XP\_001098320), *Gallus gallus* (XP\_425258), *Xenopus tropicalis* (NP\_001005789), *Takifugu rubripes* (CAB96533) and *Danio rerio* (CAK04921).



Fig. 3 3-D model of the BgMest protein

conservation between *BgMest* and the *Mest* genes of other vertebrates. But according to the analysis of development and allelic expression of the zebrafish *Mest*, Hahn et al (2005) concluded that zebrafish *Mest* gene is not imprinted, at least in the larval stage. Thus, whether the genomic structure of *BgMest* contains a typical  $C_PG$  island and structure of DNA methylation needs to be further dertermined.

According to Fig. 5, the tree may indicate the evolutionary relationships of the potential imprinting genes of mammals and other vertebrates examined in the study. The classical *Mendel's* law of inheritance suggested that both alleles are actively transcribed and functionally equivalent. However, imprinted genes represent an exception to this rule, as they are expressed



Fig. 4 Protein secondary structure of the BgMest gene





Explanations of a part of sequences used for this tree are given in Fig. 2. Branches with less than 50% support have been collapsed. *Bos taurus* (*Bos*, AAI33640); *Sus scrofa* (*Sus*, NP\_001121943); *Rattus norvegicus* (*Rattus*, NP\_001009617); *Pan troglodytes* (*Pan*, XP\_519382); *Mycobacterium tuberculosis C* (*Mycobacterium*, YP\_002076879).





1: Testis; 2: Ovary; 3: Liver; 4: Kidney; 5: Spleen; 6: Brain; 7: Stomach; 8: Lung. *Mest*-specific bands of 424bp were seen in above tissue.

from one of the two parentally inherited chromosome homologues and repressed on the other (Kaneko et al, 2002; Hou et al, 2005). The *Mest* genes of human and

#### **References:**

- Brannan CL, Bartolomei MS. 1999. Mechanisms of genomic imprinting[J]. Curr Opin Genet Dev, 9(2): 164-70.
- Charalambous M, da Rocha ST, Ferguson-Smith AC. 2007. Genomic imprinting, growth control and the allocation of nutritional resources: consequences for postnatal life[J]. *Curr Opin Endocrinol Diabetes Obes*, 14(1): 3-12.
- Edwards CA, Rens W, Clarke O, Mungall AJ, Hore T, Marshall Graves JA, Dunham I, Ferguson-Smith AC, Ferguson-Smith MA. 2007. The evolution of imprinting:chromosomal mapping of orthologues of mammalian imprinted domains in monotreme and marsupial mammals[J]. BMC Evol Biol, 7: 157.
- Georgiades P, Watkins M, Burton GJ, Ferguson-Smith AC. 2001. Roles for genomic imprinting and the zygotic genome in placental development[J]. *Proc Natl Acad Sci USA*, **98**(8): 4522-4527.
- Hahn Y, Yang SK, Chung JH. 2005. Structure and expression of the zebrafish *mest* gene, an ortholog of mammalian imprinted gene *PEG1/MEST*[J]. *Biochim Biophys Acta*, 1731: 125-132.

mouse were considered as maternal imprinted gene, and imprinting role was for methylation of C<sub>P</sub>G island (Reule et al, 1998; Kosaki et al, 2000). In mice, normal expression of Mest gene was very important for development. Knocking out and low expression of this gene may result in restraining growth and decreasing prenatal and postnatal survival (Reule et al, 1998). Nishita et al(1996)suggested that human Mest gene, the first imprinted gene to be identified on chromosome 7, was involved in the causation of Silver-Russell syndrome (SRS) (Nishita et al, 1996). Frequent loss of imprinting of the Mest gene in humans may cause cancer tissues, such as breast cancer or lung cancer and additionally, this gene regulates placental and fetal growth (Pedersen et al, 1999; Kosaki et al, 2000; Nakanashi et al, 2004; Suzuki et al, 2005). The zebrafish Mest has been found to be biallelic expressed (Hahn et al, 2005). In addition, ASCL2/CASH4 and INS were imprinted genes in mammals, but shown biallelic expression in the chicken (Yokomine et al, 2005). In the opossum (marsupial) IGF2 and M6P were imprinted as in placental mammals, but these genes are biallelically expressed in monotremes and birds (Suzuki et al, 2005). These observations may suggest that the gene imprinting occurs in mammals except monotremes (Hahn et al, 2005). Hahn et al (2005) considered that species, or clade, specific imprinted genes may have survived the selection for diluted imprinting in the evolution of non-mammalian species. Does the BgMest gene conform to the opinion of Hahn et al (2005)? Is BgMest an imprinted gene? Further research is required to uncover the structure and function of this gene.

- Hou XJ, Jiao LH, Chen X, Wang L. 2005. The effects of genomic imprinting on animal development and cloning[J]. J Genet Genomics, 32(5): 550-554. [In Chinese].
- Isles AR, Davies W, Wilkinson LS. 2006. Genomic imprinting and the social brain[J]. *Philos Trans R Soc Lond B Biol Sci*, 361(1476): 2229-2237.
- Kamei Y, Suganami T, Kohda T, Ishino F, Yasuda K, Miura S, Ezaki O, Ogawa Y. 2007. *Peg1/Mest* in obese adipose tissue is expressed from the paternal allele in an isoform-specific manner[J]. *FEBS Lett*, **581**(1): 91-6.
- Kaneko KJ, Rein T, Latham K, DePamphilis ML. 2004. DNA methylation may restrict but does not determine differential gene expression at the Sgy/Tead2 locus during mouse development[J]. *Mol Cell Biol*, 24(5): 1968-1982.
- Keverne EB, Fundele R, Narasimha M, Barton SC, Surani MA. 1996. Genomic imprinting and the differential roles of parental genomes in brain development[J]. *Brain Res Dev Brain Res*, 92(1): 91-100.

- Kosaki K, Kosaki R, Craigen WJ, Matsuo N. 2000. Isoform-specific imprinting of the human *PEG1/MEST* gene[J]. *Am J Hum Genet*, 66(1): 309-312.
- Lefebvre L, Viville S, Barton SC, Ishino F, Surani MA. 1997. Genomic structure and parent-of-origin-specific methylation of *Peg1*[J]. *Human Mol Genet*, 6(11): 1907-1915.
- Looijenga LH, Verkerk AJ, De Groot N, Hochberg AA, Oosterhuis JW. 1997. H19 in normal development and neoplasia[J]. *Mol Reprod Dev*, 46(3): 419-439.
- Mezquita J, Mezquita PM. 1997. Characterization and expression of two chicken cDNAs encoding ubiqutin fused to ribosomal protein of 52 and 80 amino acids[J]. *Gene*, **195**: 313-319.
- Nishita Y, Yoshida I, Sado T, Takagi N. 1996. Genomic imprinting and chromosomal localization of the human *MEST* gene[J]. *Genomics*, 36: 539-542.
- Nakanashi H, Suda T, Katoh M, Watanabe A, Igishi T, Kodani M, Matsumoto S, Nukamoto M, Shigeoka Y, Okabe T, Oshimura M, Shimizu E. 2004. Loss of imprinting of PEG1/MEST in lung cancer cell lines[J]. Oncol Rep, 12(6): 1273-1278.
- Pedersen JS, Dervan PA, Broderick D, Harrison M, Miller N, Delany F, O' Shea D, Costello P, McGoldrick A, Keating G, Tobin B, Gorey T, McCann A. 1999. Frequent loss of imprinting of PEG1/MEST in invasive breast cancer[J]. *Cancer Res*, **59**(21): 5449-5451.

Pedersen JS, Dervan P, McGddrick A, Harrison M, Ponchel F, Speirs V,

Isaacs JD, Gorey T, McCann A. 2002. Promoter switch: a novel mechanism causing biallelic PEG1/MEST expression in invasive breast cancer[J]. *Hum Mol Genet*, **11**(12): 1449-1453.

- Reule M, Krause R, Hemberger M, Fundele R. 1998. Analysis of Peg1/Mest imprinting in the mouse[J]. Dev Genes Evol, 208(3): 161-163.
- Saitoh S, Wada T. 2000. Parent-of-origin specific histone acetylation and reactivation of a key imprinted gene locus in Prader-Willi syndrome[J]. Am J Hum Genet, 66(6): 1958-1962.
- Scott RJ, Spielman M. 2004. Epigenetics: imprinting in plants and mammals-The same but different?[J]. *Curr Biol*, 14: 201-203.
- Suzuki S, Renfree MB, Pask AJ, Shaw G, Kobayashi S, Ishino TK, Ishino F. 2005. Genomic imprinting of IGF2, p57(KIP2) and PEG1/MEST in a marsupial, the tammar wallaby[J]. *Mech Dev*, 122: 213-222.
- Yokomine T, Shirohzu H, Pubowasito W, Toyoda A, Iwama H, Ikeo K, Hori T, Mizuno S, Tsudzuki M, Matsuda Y, Hattori M, Sakaki Y, Sakaki H. 2005. Structural and functional analysis of a 0.5-Mb chicken region orthologous to the imprinted mammalian Ascl2/Mash2-lgf2-H19 region[J]. *Genome Res*, 15: 154-165.
- Zai Y, Qu XH, Zhou GQ, He FC. 2002. Cloning and tissue expression of the novel human differentiation-related gene Ndr2[J]. Chn J Biochem Mol Biol, 18(2): 139-144. [In Chinese].