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金钱鱼促性腺激素受体基因克隆及其在性腺发育 不同时期mRNA表达水平的分析

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摘要: 为了解金钱鱼促性腺激素受体基因(GtHRs)在金钱鱼性腺发育中的作用,采用反 转录PCR (Rt-PCR)与cDNA末端快速克隆(RACE)首次克隆了金钱鱼卵泡刺激素受体 (FSHR)和促黄体生成素受体(LHR)的cDNA序列全长。FSHR的cDNA全长2538 bp, 编码 702个氨基酸,LHR的cDNA全长3315 bp,编码722个氨基酸,它们都含有属于糖蛋白激 素受体(GHR)家族的典型跨膜螺旋结构区域(TM helix)。同源性分析显示金钱鱼GtHRs与 欧洲鲈的相似性最高,且GtHRs在进化中具有一定的保守性。性腺不同时期表达分析表 明,在卵巢Ⅰ期时,FSHR高水平表达,Ⅱ、Ⅲ、Ⅳ期时在较低水平表达。在精巢中, FSHR的表达水平在Ⅰ、Ⅱ、Ⅲ期逐渐升高并在Ⅲ期达到最高、Ⅳ期开始下降。LHR在 金钱鱼卵巢和精巢的Ⅰ、Ⅱ、Ⅲ期低水平表达,在Ⅳ期表达水平达到最高。研究表明, FSHR在金钱鱼性腺发育早期扮演重要作用,LHR与卵子和精子的成熟有关。 关键词:金钱鱼;卵泡刺激素受体;促黄体生成素受体;序列分析;基因表达 中图分类号: O 785; S 965

在脊椎动物中, 配子的形成主要由脑—垂 体—性腺轴(brain-pituitary-gonad, BPG)调控。垂 体分泌的促性腺激素(gonadotropic hormone, GtHs)包括卵泡刺激素(follicle-stimulating hormone, FSH)和促黄体生成素(luteinizing hormone, LH), 它们作用于性腺,诱导调控配子形成^[1]。FSH和 LH功能的实现依赖于和它们的受体结合,启动 下游信号传导通路, 致使性类固醇生成相关蛋 白,诱导性激素合成^[2]。这种调控机制也存在于 硬骨鱼类中^[3]。促性腺激素受体(gonadotropic hormone receptor, GtHRs)包括卵泡刺激素受体 (follicle-stimulating hormone receptor, FSHR)和促黄 体生成素受体(luteinizing hormone receptor, LHR),属于G蛋白偶联受体超家族糖蛋白,由胞 外区、胞内区及跨膜区3部分构成[4]。二者在鱼 类生殖发育周期的配子发生过程中的特定时期

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发挥着功能。在雌鱼体内, FSHR与卵黄生成有 关,而LHR则促进卵母细胞的成熟和排卵^[5-6]。真 鲷(Pagrus major)的FSH mRNA在卵巢发育过程中 则始终维持在较低状态, LH mRNA在两性配子 发生到排出始终保持较高水平^[7]。牙鲆(Paralichthys olivaceus)体内两受体GtHs的转录水平均随卵 巢发育逐渐升高,并在卵子成熟阶段达到最高。 在雄鱼体内, GtHRs与促进精巢成熟有关, 影响 精子发生和排出^[8]。在繁殖初期的虹鳟(Oncorhynchus mykiss)和大西洋鲑(Salmo salar)体内, FSHR 基因mRNA的表达量变化不显著,随着精子发生 而显著升高,在排精时达到最大表达量[9-10]。在 斑马鱼(Danio rerio)体内, LHR基因mRNA也是在 排精时达到最大表达量^[11]。这些结果表明GtHs在 硬骨鱼生殖调控中的功能存在种属特异性。 GtHs的作用是通过结合GtHRs实现的,因此研究

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GtHRs在配子形成过程中的表达和调控,可更深入理解GtHs的作用机制。

金钱鱼(Scatophagus argus)属鲈形目(Perciformes),为广盐性亚热带鱼类,是我国东海和 南海较常见的优质经济鱼类。过度捕捞导致野 生鱼苗资源量减少,远远不能满足需求,迫切 需要人工繁殖。在人工繁殖过程中极易出现金 钱鱼雌雄个体性腺发育不同步的现象,导致繁 殖不成功,给人工繁育金钱鱼带来极大的困难^[12]。 目前国内外对金钱鱼生长繁育方面的研究比较 少,有关金钱鱼性腺发育尤其是配子形成的分 子机制还未阐明,因此开展其分子机理的研究 具有重要意义。

本研究以人工养殖金钱鱼为实验材料,通 过组织学切片对金钱鱼的性腺发育时期进行了 鉴定。采用RACE技术克隆得到金钱鱼FSHR和 LHR基因cDNA序列,应用Rt-PCR技术对FSHR和 LHR mRNA在不同组织的表达及在性腺不同发育 时期的表达进行研究。为进一步阐明FSHR和 LHR的生理功能和分子机制奠定基础,同时也为 金钱鱼生殖调控及育种提供基础数据。

1 材料与方法

1.1 实验对象

实验用金钱鱼取自广东龙胜鱼苗繁殖有限 公司养殖基地。生长发育4个时期,分别取每个 时期雌鱼和雄鱼各5条的性腺混合作为一个样, 共40条鱼。利用液氮迅速冷冻样品,然后置于 -80°C冰箱保存待用。

1.2 总RNA的提取和cDNA合成

将样品放入液氮中研磨。总RNA的提取使 用Trizol Reagent试剂盒,按说明书提取RNA。用 琼脂糖电泳检测RNA的完整性,核酸蛋白定量 (nanodrop 2000)测定RNA浓度,然后-80°C保存 备用。以总RNA为模板,cDNA第一链利用Oligo (T)d16来合成,获得cDNA用于克隆实验。按照 PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa)操作说明进行反转录用于荧光定量,反 转录cDNA均稀释10倍。合成的cDNA于-20°C保 存备用。

1.3 FSHR和LHR序列克隆

根据金钱鱼转录组数据库序列设计引物(表1)

并验证此中间片段, PCR反应体系为25 μ L, 含2.5 μ L 10×Buffer、2.5 μ L MgCl₂、1 μ L dNTP (2.5 mmol/L)、上游和下游引物各0.5 mL (10 μ mol/L)、 0.3 μ LTaq酶(5 U/ μ L)和1 mL模板, 以ddH₂O补充 至25 μ L。扩增条件: 94 °C 3 min; 94 °C 45 s, 58 °C 30 s, 72 °C 1 min 30 s, 30个循环; 72 °C延伸10 min。 PCR产物切胶纯化回收后,连接到pMD18-T (TaKaRa)载体,转化大肠杆菌DH5 α 感受态细 胞,经鉴定后将阳性克隆送上海生工生物工程 有限公司测序。

根据已得到中间片段序列设计3'RACE引物 (表1), 3'FSHR-1、3'FSHR-2、3'RACE的操作 参照TaKaRa Prime Script Reverse Transcriptase试剂 盒推荐方法进行。首先以试剂盒提供的3'RACE Adaptor为引物进行反转录,然后以试剂盒提供 的AP和按要求设计的3'FSHR-1进行第1轮PCR扩 增。反应条件: 94 °C 3 min; 94 °C 30 s, 55 °C 30 s, 72°C 2 min, 30个循环; 72°C延伸10 min。以第 1轮PCR产物为模板, 3'RACE-R和3'FSHR-2为 引物进行第2轮PCR扩增。反应条件: 94 ℃ 3 min; 94°C 30 s, 60°C 30 s, 72°C 2 min, 35个循环; 72 °C 10 min。将获得的扩增产物连接到pMD18-T载体,转化大肠杆菌DH5α感受态细胞,挑取阳 性克隆送上海生工生物工程有限公司测序。5'R-ACE参照Hu等^[13]方法,设计5'RACE引物(表1), 首先以AOLP为引物进行反转录得到cDNA第 1链,用RNaseH消化cDNA,然后用末端脱氧核

表1 实验用引物序列

Tab. 1 Primer sequences for PCR study

引物 primer	序列 sequence (5'-3')
AP (Adaptor Primer)	CTGATCTAGAGGTACCGG ATCC
Oligo (dT)16AP	CTGATCTAGAGGTACCGGATCCTTTTTTT TTTTTTTT
AOLP	GGCCACGCGTCGACTAGTACTTTTTTTT TTTTTTV
3'RACE-R	CTAGAGGTACCGGATCCTT
5'FSHR-1	GGAGCAGTGGTCCCTGTAGAA
5'FSHR-2	CGTTGGGATTGATGTGAGTAAG
3'FSHR-1	GGTCCTCTTCTACCCGATCAAC
3'FSHR-2	ACAGATTTACCGGACAGAGAGTTC
5'LHR-1	GTACAAGTTGTCACAGATATCCAAGAT
5'LHR-2	ATTGTGTTGGAGAAGCAGCGGC
3'LHR-1	TGAAGCAGATGCTTTCAATCCCT
3'LHR-2	CAGACTTCTGCATCGGGATCTAC

苷转移酶(TaKaRa)进行5′端加polyA尾得到5′RACE 模板。以Oligo (dT)16AP和5′FSHR-1为引物进行 第1轮PCR,反应条件为94 ℃ 3 min;94 ℃ 30 s, 56 ℃ 30 s,72 ℃ 1 min 30 s,30个循环;72 ℃ 10 min。然后以第1轮PCR产物为模板,5′FSHR-2和AP为引物进行第2轮PCR,反应条件:94 ℃ 3 min;94 ℃ 30 s,60 ℃ 30 s,72 ℃ 1 min 30 s, 35个循环;72 ℃ 10 min。PCR产物切胶纯化回 收、连接、测序、拼接。LHR基因克隆同FSHR。

1.4 序列分析

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用DNAStar软件包中的SeqMan将FSHR和 LHR的中间片段、3'RACE和5'RACE测序所得序 列进行拼接得到FSHR和LHR的全长cDNA序列。 在NCBI中BLAST (http://www.ncbi.nlm.nih.gov/BLAST) 进行同源性分析,并使用ORF Finder (http://www. ncbi.nlm.nih.gov/gorf/orfig.cgi)确定开放阅读框 (open reading frame, ORF)。利用在线工具TMH-MMServer 2.0(http://www.cbs.dtu.dk/services/TMHMM) 对蛋白的跨膜区域进行预测;利用在线工具 SignalP 4.1 Server (http://www.cbs.dtu.dk/services/ SignalP)预测信号肽;利用ProtParam (http://web. expasy.org/protparam)对所推测的蛋白质基本物理 化学参数进行分析;利用NetPhos 2.0 Server (http:// www.cbs.dtu.dk/services/NetPhos)预测Ser、Thr和Tyr 磷酸化位点。利用NetNGlyc1.0 Server (http://www.cbs. dtu.dk/services/NetNGlyc)预测N端糖基化位点。利 用Mega 6.0 软件采用邻位相连法Neighbor-Joining 结合Clustal X软件构建分子系统进化树。

1.5 金钱鱼卵巢、精巢不同发育时期组织学 染色

样品固定后经梯度酒精脱水,二甲苯透明, 石蜡包埋,石蜡切片厚度5~6μm,H.E染色, Nikon显微镜拍照并观察。卵巢和精巢发育分期 参照崔丹等^[14]的方法。

1.6 金钱鱼*FSHR*和*LHR*基因在雌雄不同时期的表达分析

采用ABI 7500荧光定量PCR仪(Applied Biosystems, USA)。PCR反应体系(共10 μL): SYBR Premix Ex Taq II(2×) 5.0 μL, 上、下游引 物(10 μmol/L)各0.2 μL, ROX II(50×) 0.2 μL, cDNA模板1.0 mL, 用水补至10 μL。反应条件: 95 °C预变性30 s; 95 °C变性5 s, 55 °C退火34 s, 40个循环; 95 ℃变性15 s, 60 ℃退火1 min, 95 ℃ 延伸15 s。实时荧光定量数据以2^{-ΔΔCT}法处理,表 达量变化以mean±SD表示。为确保实时荧光定量 PCR反应的准确性和可重复性,每个样品均重复 测定3次。使用SPSS软件进行方差分析和多重比 较,显著性水平设为P<0.05。

2 结果与分析

2.1 金钱鱼FSHR和LHR基因序列分析

金钱鱼FSHR的GenBank登录号为KU646840, cDNA全长2538 bp, 开放阅读框(ORF)2109 bp, 共编码702个氨基酸。预测金钱鱼FSHR蛋白分子 量为78.255 ku,理论等电点为8.84。该氨基酸序 列分为377 aa的胞外区域(ECD),其中20 aa为信 号肽;长度为263 aa的7次跨膜螺旋区(7 TM helices),其中3个胞内环(IL)和3个胞外环(EL)连 接这7个跨膜螺旋区;有62 aa胞内区。预测整个 编码区有17个Ser磷酸化位点、5个Thr磷酸化位 点和3个Tyr磷酸化位点。推测该氨基酸含有5个 潜在的N基端糖基化位点,分别为⁶²NTTC、 ²³²NGTK、³⁰⁹NLTY、³²⁵NRSR、³⁵¹NSTS(图1)。 LHR的GenBank登录号为KU886226, cDNA全长 3315 bp, 开放阅读框(ORF)2169 bp, 共编码 722个氨基酸。预测LHR蛋白分子量80.185 ku, 等电点8.64。该氨基酸序列分为389 aa的胞外区 域(ECD),其中包含21个信号肽;长度为263 aa 的7次跨膜螺旋区(7 TM helices),其中3个IL和3个 EL连接这7个跨膜螺旋区;长度为70 aa胞内区。 预测整个编码区有14个Ser磷酸化位点、4个 Thr和6个Tyr磷酸化位点。推测该氨基酸含有4个 N基端糖基化位点,分别为⁴²NLTQ、¹⁹⁹NGTK、 ³⁰³NGST和⁴⁰³NLTV(图2)。

2.2 FSHR和LHR氨基酸同源性分析

利用CLUSTAL X在线软件对金钱鱼的氨基 酸序列进行对比,金钱鱼FSHR与欧洲鲈 (Dicentrarchus labrax)和点带石斑鱼(Epinephelus coioides)FSHR同源性较高,分别为91%和80%, 与斑马鱼同源性为60%,与人类等哺乳动物同源 性为51%(表2)。金钱鱼LHR同样与欧洲鲈和点带 石斑鱼LHR同源性较高,分别为89%和87%。与 斑马鱼同源性为69%,与人类等哺乳动物同源性 为52%~53%(表3),FSHR与LHR在进化中比较保守。 不同物种氨基酸序列对比发现了GpHR家族

1 GGAAACTGCCTGCAGGCTGCAGACGGGACTGTTCAAAACACGCCCACTAGCTGGCACACCTGACAATCAACAGAACAACTAAGT 85 AAATTATTGACTGACTTCAGGTGAGAGAGTGTGAGCAGACTCAGAAGTAGAATAAGAGCAGAAGCGAACCTGGACTGCGCGAGACTAGG 175 ATGATGATGGTCATGATTCTCATAATGTTCATGATGCTGATGATAAAGACGGCAACAGCCTCGGTGCCTGGCCCAGAGATGCACGTTAAA 1 <u>M M M V M I L I M F M M L M I K T A T A</u> S V P G P E M H V K 265 CCAGGAGTGGAGACCAGCTTGGCCATACGAACCCTGAGCTTCAGCTACCAGTTGAAGTTTGGGGTCACAGAGATTGCCTCCAGCATCCCC 31 P G V E T S L A I R T L S F S Y Q L K F G V T E I A S S I P 355 AGCAACACCACATGCCTGGTAGTTAAGCAGACGGAGGTCGTAGTGATTCCCCAGGGCGCGCTCAACAGCCTGCAGCACCTCAGGAAAGTC S N T T C L V V K Q T E I V V I P Q G A L N S L Q H L R K V 61 445 ACCATATGGGAGAACGACAAGCTGGAGAGTATCAATGAGTTTGCCTTCGCCAGCCTCTCCAGCTCACCGATTTCTTCATCTCGGGAAT 91 T I W E N D K L E S I N E F A F A S L S Q L T D F F I S G N 535 GTTGCGTTAAAAAATATAGGAGCTTTTGCTTTCTCTGAGCTGCCTGAACTCACTGAGATAACCATAACAAAGTCAGAACACCTGACACAC 121 VALKNIGAFAFSELPELTEITITKSEHLTH 625 ATCAATCCAGATGCATTCAAGGACATTGTGAAACTGAAGTATCTGACCATCGCCAACACTGGACTGAGGGTTTTTCCAGACTTCACCAAG 151 INPDAFKDIVKLKYLTIANTGLRVFPDFTK 181 I H S T G V L L F D L H E N S H I E R V P A N A F R G L C S 805 CAAACCATCACAGAGATACGGCTCACCAGAAATGGCATCAAGGAGGTGGCAAGTGACGCCTTCAACGGCACAAAGATGCACAGATTGTTC 211 QTITEIRLTRNGIKEVASDAF<mark>NGTK</mark>MHRLF 895 CTAGGAGGCAACCAGCAGCTTACTCACATCCAATCCCAACGCCTTTGTGGGTTCCAGTGAGTTGGTGGTACTGGACATTTCCCACACAGCC 241 LGGNQQLTHINPNAFVGSSELVVLDISHTA 985 CTCAGCTCCCTGCCAGACTACATCCTTGGTGGTCTGCAGAAGCTGATCGCAAAGTCCGCCTTCCATCTGAAGGAACTTCCTCCTCGCAG 271 LSSLPDYILGGLQKLIAKSAFHLKELPPLQ 1075 CTCTTCTCCAAACTCCACCAGGCCAACCTGACGTACCCATCACACTGCTGTGCCTTCCAAAACATACACAGGAACAGATCAAGGTGGAGC 301 LFSKLHQANLTYPSHCCAFQNIHRNRSRWS 1165 TCCCTGTGCTCCCACCCAAAGGCTCCAGATAACCTGCACTTCTACAGGGACCACTGCTCCAACTCTACCTCCATCACTTGTACTCCGACC 331 SLCSHPKAPDNLHFYRDHCS<mark>NSTS</mark>ITCTPT 1255 CAAGACGATTTCAACCCCTGTGAGGACATCATGTCCCCTGTGCCCCTACGGGTCCTCATCTGGATTGTCTCCATCCTGGACTGCTGGGG 361 QDDFNPCEDIMSPVPLRVLIWIV S Ι 1345 AACGCAGCGGTTCTTCTGGTCCTGTTAGGCAGCCGCCCCAAACTGACCGTCCCCCGTTTCCTCATGTGCCACTTGGCCTTTTCTGACCTC NAAVLLVLLG Serskltvp 391 R F L 🔊 C H L 1435 TGCATGGGCATCTACCTAGTAGTCATAGCAACTGTAGACATGCTCACTCGTGGTCGGTACTACAACCACGCCATAGACTGGCAGACGGGC 421 CMGIYLVVIATVDML TRGRYYNH D W Q A 1525 CTGGGCTGCAGCGCTGCAGGCTTCTTCACGGTGTTTGCCAGTGAACTGTCAGTGTTCACACTAACAGCAATCACCCTGGAGCGCTGGCAC 451 <u>LGCS</u>AAGFFTVFASELSVFTLTAITLE W H 481 TITHALRL D R K L R L R L A C I V M T A G W I F S S L 1705 TCTGCTTTGTTACCCACACTTGGGGTCAGCAGCTACAGCAAGGTGAGTATCTGTCTTCCCATGGACGTGGAGTCTCTGGTGTCTCAGGTC 511 SALLPTLGV<u>SSYSKVSICLPMDVES</u> 1795 TATGTGGTCTCTCACCTCCTCAACATCCTGGCCTTCTTCTGCGTGTGCTGCTGCTGCTACCTCAGCATCTACCTCACCGTCCGCAACCCC 541 Y V V S L L L N I L A F F C I Y L 1885 TCGTCAGCGCCGGCCCACGCCGACACCCCGCGTGGCCCAACGCATGGCCGTCCTCATCTTTACCGACTTCCTGTGCATGGCTCCCATATCC SSAPAHADTRVAQR**V**AVLIFTDFL 1975 TTCTTTGCCATCTCAGCGCCCCTCAAGCGTCCTCTCATCACCATCTCAGATGCCAAACTCCTGCTGGTCCTCTTCTACCCGATCAACTCC FFAISAAL<u>KRPLITISD</u>AKLLLVLF 601 C S N P F L Y A F F T R T F R R D F F L L A A R F G L F K T 631 2155 CGAGCACAGATTTACCGGACAGAGAGTTCCTCCTGTCAGCAGCCGGCATGGACCTCTCCTAAGGGCAGCCATGTGATGCTGTACTCCTTG 661 RAQIYRTESSSCQQPAWTSPKGSHVMLYSL 2245 GCCAATACGCTGAGTGTCGATGGGAAACATGAGTACTGAGTCGTCATTTAAAAAGAGATGAAGACGTTTTACCGGATTGTGATCAGGAGGT 691 ANTLSVDGKHEY* 2335 CTCCTCACAGAAGTCCTCTTAGGTCTATGGGGATAGCCAGAGTCTAATTCATGAACATCTCGTAATGTTGTAAGAAACTTTGGTGCAATG

2425 AGAGATGTACTCTGTATATACAGTGTCATAGGAAATTCAAGTAAATATGGTACAGAACAGCTGAAAATACTAGATTAAAAAAATTTGGTGCAATG 2515 CTGTCCTGGATAAAAAAAAAAAAAAA

图 1 金钱鱼FSHR cDNA全长序列

MMMVM: 信号肽; VLIWIV: 阴影部分跨膜螺旋区; 黑色线条: 胞内环; 黑色箭头: 胞外环; 方框: 潜在的N端糖基化位点; ATG: 起始密码子; TGA: 终止密码子; 下同

Fig. 1 Full sequence of FSHR of S. argus

MMMVM: signal protein; VLIWIV: shadows are transmembrane helices; black lines: intracellusar loops; black arrows: extracelluar loops; squares: potential N-linked glycosylation sites; ATG: initiation codon; TGA: termination codon; the same below

张 逛,等:金钱鱼促性腺激素受体基因克隆及其在性腺发育不同时期mRNA表达水平的分析

7期

GGTTAGCCTCCCGGTGCTGCAGGCTCTGGTTCTGTCTCTTAG 1 43 133 ATGTGGACGTCTCTGCCAGTCCTCTTGCCCCTAATCGTTTTAGTCTTCTCCCGGGTGCAAGAGCGCTTCGAAGTCGCCTGTCCGAGGATC <u>M_W_T_S_L_P_V_L_P_L_I_V_F_S_G_C_K_S</u>_A_S_K_F_A_C_P_R_I 223 TGCCGCTGCTTCTCCAACACAATCAGATGCAACAATTTGACACAAGGGTCTGCGCGGATGATGGACCACAGAGATAAAAGGCTGTTTTTC C R C F S N T I R C N N L T Q G S A R M M D H R D K R L F F 313 TATCACTTGTCTTTACACACTATCTCAAGCCGTTCTTTTGAGGGGTTGAAAGGAGTCCAGAGAATAGAGATTGCTCAGAGCGTGACCCTG Y H L S L H T I S S R S F E G L K G V Q R I E I A Q S V T L 403 GAAACCATTGAGACATTAGCGTTTAATAACCTTCTCAGCCTTTCTGAAATCTCAATCCAGAACACCAGGAGCCTGATGCACATTGGCAGA E T I E T L A F N N L L S L S E I S I Q N T R S L M H I G R 493 AGGACATTTAACAACCTTCCTAAACTGCATTATTTGAGCATCTCAAATACTGGGTTGACAGTTTTTCCTGATATTACGTCGATTAATTCT R T F N N L P K L H Y L S I S N T G L T V F P D I T S I N S 583 CTTGAATCAGAGTTTATCTTGGATATCTGTGACAACTTGTACCTACTGGAAATACCTCCAAACGCTTTCATTGGAATGACGAAAGAATAT L E S E F I L D I C D N L Y L L E I P P N A F I G M T K E Y V T M N M Y N N G I R E I H D H A F <mark>N G T K</mark> I D K L V L K N 763 AATCGAAAACCTCAGAGTGATCCACAGAGATGCCTTCAAAGGAGCCCACAGGCCCTGGAGTCTTGGACGTGTCTGCCACAGCTCTCACAAAG N R N L R V I H R D A F K G A T G P G V L D V S A T A L T K L P A Q G L E S V L V L L A Q S A Y A L K S L P P L Q G L W S L R E A H L T Y N S H C C A L L S W N T D R D L P I H P A 1033 GGGAATAATGGCTCTACATATTGTGATGAGAGTGACCCATCAGCAAGGGTTCAGCATGTGATTGGAGGTTCAGCAGACATGACCTTTGTC G N N G S T Y C D E S D P S A R V Q H V I G G S A D M T F V 123 AAAGATATGCCATTTTTTTCAGACGTCAATCTGTTTATGGAAGATGAAGGCTTTGGAGATGTGAATTTCCACTATCCAGAACTAGACCTC K D M P F F S D V N L F M E D E G F G D V N F H Y P E L D L 1213 TGTCAGACCCAACCGACTTTGGTTTGTACACCTGAAGCAGATGCTTTCAATCCCTGTGAGGACATCGCAGGGTTCAGTTTTCTCAGAGTG C Q T Q P T L V C T P E A D A F N P C E D I A G F S F L R V 1303 GCCATTTGGTTTATCAACATACTGGCCATCACAGGTAACCTGACAGTCCTCCTGGTCTCCTTCGCCAGCCGGAACAAGCTGACCGTGTCT AIWFINILAITG<mark>NLTV</mark>LLVSFA<mark>S</mark>RNKL**T** V S 1393 CGTTTTCTCATGTGCCACCTGGCTTTTGCAGACTTCTGCATCGGG ATCTACCTCTTGATGATAGCCACAGTAGACTTGCGCACACGTGGT R F L M C H L A F A D F C I G I Y L L M I <u>A T V D L R T R G</u> <u>HYSQHAIEWQ</u>TGPGCSAAGFLSVFGGELSV 1573 TACACGCTTTCCACCATCACCCTGGAGCGCTGGCACACCATCACCAATGCTCTGCGGATAGGGCGACATCTGGTACTCACGCAGGCGGCG YTL<mark>Ş</mark>TITLERWHTITNALRIGR<mark>Y</mark>LVLTQAA 1663 AGTATAATGCCGACTGGATGGCTCATCTGTCTGGGGATGGGGATGCGGCATGCGGTGGGGTGGGGGGGTGTGGGCAGTTATGCCAAAGTGAGCATGTGC SIMATGWLICLGMGML<u>PLVGVSSYAKVSMC</u> <u>PMDIETPLAQAFI</u> IIILLFNVGAFVVVCV 1843 TGTTATGTGCTGATCTATCTGGCTTTAAAGAACCCTGAGTTACCCAGAAGAAGTGCTGACGCCAAGATTGCAAAGCGCATGGCCGTGCTC CYVLIYL 🗛 LKNPELPRRSADAKIAKRU AVL 1933 ATCTTCACAGATTTTCTCTGCATGGCGCCCATCTCCTTCTTTGCCATCTCGCAGCGTTCAAGGTTCCCCTCATCACAGTTACCAACTCC I F T D F L C M A P I S F F A I S A A F K V P L I T V T N S 2023 AAGATTCTGCTGGTCCTCTTCTCCCCATCATTCTTGTGCCAATCCTTTCCTTTACGTCATCTTCACCAAGGCTTTCAGAAAGGATGCA KILL VLFFPIN SCANPFLYVIFTKAFRKDA 2113 TATCAGCTCATGAGTGCCATGGGCTGCTGTAAAAGCAAGGCCAGGATTTATCGCATGAAGGCCTACTATGGCAAAAATGCATTCAAGAAC Y Q L M S A M G C C K S K A R I Y R M K A Y Y G K N A F K N N L G S S N K R S S P G V R L A V M A G Q S H H V K E E V E 2293 CTGACC**TGA**AATAAGAAAAAGAAATGCAAAATTCCTTTAGACTATTAGGACGGAAGATCAAGGAAATTGAGGAAATAAGAAGCTGGATTTGG L T * 2653 CAGTCCCACCTGTAGCTTCCCTGCTTATCACGTTTCCTTTGCAAAAGTCCTATGGTGAGGTGAACATTAAGAAAAATTTGCAGAGGCAAA 2833 ATGGACAAAAAGTCCTTAAAAACACTTCTATGATAAACTGAGACTGAGTGATCACCTTCATCCTGCCTTCATCTCCAAGCAGTTGTTGTTG 2923 TTTTATTGCAGTGAAAAACCAAACTATAGCCGCCAGTTTTTTCCCAGCCCTGTTATAAAAATGAAACTGTTTTATTCTGAGGTTGGCAGCGT 3013 ATGAGGTTGTCTAAAATTTATTAAGAATATCGTGACTAATATGAAAAACCAAGGAGTCTGACCACGTGGTGGAGTAGTTGGGAAGGCGACCA 3103 GGCGACTACATCACCAGGTTGGTGTAAGGATTTTCCTTTAAGATTGTGTTACCAAAACGCGAAGAGATGAAACACGTCTGTGTTGGTTACA 3193 TACTTGTGTATATTACTGTAACTTTTGTTTGTGTTGGCCGAACATTTTTCTGCCTAAAAAAAGGCAAAAACATAGGAGAAATAAAATTATT 3283 CCAAGGAAAAAAAAAAAAAAAAA

图 2 金钱鱼LHR cDNA全长序列

Fig. 2 Full sequence of *LHR* of *S. argus*

997

表 2 FSHR氨基酸序列的同源性比对

amino acid sequence of FSHR

物种(GenBank登录号)	同源性/%
species (GenBank accession no.)	identity
人 Homo sapiens (AAB26480.1)	51
小鼠 Mus musculus (NP_038551.3)	51
大鼠 Rattus norvegicus (NP_954707.1)	51
矮黑猩猩 Pan paniscus (XP_003822740.1)	51
绵羊 Ovis aries (AAA31525.1)	51
马 Equus caballus (NP_001157485.1)	51
原鸡 Gallus gallus (NP_990410.1)	51
美洲洞蛇 Bothrops jararaca (AAO72730.1)	51
黄颡鱼 Tachysurus fulvidraco (AEH84420.1)	58
大西洋鳕 Gadus morhua (ABD62885.1)	67
大西洋鲑 Salmo salar (ABH10576.1)	67
青鳉 Oryzias latipes (BAJ05402.1)	77
斑马鱼 Danio rerio (AAR84280.1)	60
虹鳟 Oncorhynchus mykiss (AAQ04551.1)	66
点带石斑鱼 Epinephelus coioides (AIW52567.1)	80
欧洲鳗鲡 Anguilla anguilla (CPR30242.1)	59
欧洲鲈 Dicentrarchus labrax (AAV48628.1)	91

保守信号序列为CCAF、ERW、FTD、NPFLY (图3, 4)。

利用MEGA6.0构建系统进化树,FSHR和 LHR进化树分析发现,进化树可分为两支,其中 各种鱼类聚为一支,哺乳类、两栖类和鸟类聚 为一支。金钱鱼FSHR和LHR均处于鱼类这一支 且均与欧洲鲈亲缘关系最近(图5,6)。

2.3 金钱鱼卵巢、精巢不同发育时期组织学观察

金钱鱼卵巢和精巢发育可分为4个时期。卵 巢Ⅰ期卵母细胞分化,Ⅱ期卵母细胞发育出现 卵黄核,Ⅲ期卵母细胞出现脂肪油滴,Ⅳ期卵 母细胞发育成熟,含大量卵黄颗粒。精巢Ⅰ期 精原细胞增殖,Ⅱ期精母细胞生长发育,Ⅲ期 精母细胞分化成精子细胞,Ⅳ期精子发育成熟。

2.4 金钱鱼不同发育时期性腺中FSHR和 LHR基因mRNA的表达水平

根据H.E染色结果可将卵巢和精巢分为Ⅰ、 Ⅱ、Ⅲ、Ⅳ期(图7)。Real-time PCR结果(图8)显

表 3 LHR氨基酸序列的同源性比对

Tab. 3Comparative identity of

amino acid sequence of LHR

物种(GenBank登录号)	同源性/%			
species (GenBank accession no.)	identity			
小鼠 Mus musculus (AAA39432.1)	53			
大鼠 Rattus norvegicus (AAA41529.1)	52			
人 Homo sapiens (AAB19917.2)	52			
原鸡 Gallus gallus (BAA23736.1)	54			
家牛 Bos taurus (AAM09535.1)	52			
黄颡鱼 Tachysurus fulvidraco (AEH16748.2)	50			
大西洋鳕 Gadus morhua (ABD62886.1)	49			
大西洋鲑 Salmo salar (ABH10577.1)	71			
青鳉 Oryzias latipes (BAJ05403.1)	49			
斑马鱼 Danio rerio (AAR84281.1)	69			
草鱼 Ctenopharyngodon idella (ABM73668.1)	68			
虹鳟 Oncorhynchus mykiss (NP_001117798.1)	70			
底鱂 Fundulus heteroclitus (BAF48337.1)	76			
点带石斑鱼 Epinephelus coioides (AEG65827.1)	87			
欧洲鳗鲡 Anguilla anguilla (CPR30244.1)	61			
欧洲鲈 Dicentrarchus labrax (ABX79919.1)	89			

示*FSHR*基因mRNA在卵巢中 I 期高水平表达, Ⅱ、Ⅲ、Ⅳ期显著下降(*P*<0.05),但Ⅱ、Ⅲ、 Ⅳ期表达水平逐渐升高,Ⅳ期相对于Ⅱ期有显 著性升高(*P*<0.05)。随着精巢发育,*FSHR*基因 mRNA表达水平逐渐升高并在Ⅲ期达到最高,在 Ⅳ期显著性降低(*P*<0.05)。*LHR*基因mRNA在卵巢 和精巢的 I、Ⅱ、Ⅲ期表达水平较低,且 I、 Ⅱ、Ⅲ期之间无显著性变化(*P*>0.05);在Ⅳ期, *LHR*基因mRNA的表达相比 I、Ⅱ、Ⅲ期均有显 著提升(*P*<0.05)。

3 讨论

本研究通过Rt-PCR和RACE技术克隆了金钱 鱼FSHR和LHR基因的cDNA序列全长,分别编码 702和722个氨基酸。金钱鱼FSHR和LHR基因编码 的氨基酸均与欧洲鲈同源性最高,分别为92%和 89%;FSHR和LHR与斑马鱼同源性分别为60%和 69%。金钱鱼GtHRs氨基酸序列具有GHRs家族典 型的7次跨膜螺旋结构(7 TM helices)^[15]。系统发 育分析也显示金钱鱼GtHRs与鲈形目聚为一支,

	Sa Dl Ea Ol Gm Tf Dr Aa	Signal peride Signal peride MHWYMILINHMIMIKTATASVPOPEMHVKPGVETSLAIRTLSFSYQLKFGVTEIASSIPSNTTCLVVKQTEIVVIPQGALNSICH_RKVTIWENDKLESINEFAFASLSQLTDFFISGN MHWYMILINHMIKTATASVPOPEMDVKPGVETSLAKRTLSFCYQLKFGVTEISSISSNTTCLVVKQTEIVVIPQGALNSICH_RKLTIWENDKLESINEFAFASLSQLTDFFISGN MHMIMILINHMIKTATASVPOPEMDVKPGVETSLAKRTLSFCYQLKFGVTEISSISSNTTCLVVKQTEIVVIPQGALNSICH_RKLTIWENDKLESINEFAFASLSQLTDFFISGN MHMIMILINHMIKTATASVPOPEMDVKPGVETSLAKRTLSFCYQLGFGUFESSISSNTTCLEVKQTEIVVIPQGALNSICH_RKLISKDMLESINEFAFASLSQLTDFFISGN MMMIMILINHMIKTAASPOPEMDVKPGVETSLAKRTLSFCYQLGFGUFESSISSNTTVNIKGTIVIPQGALTSICH-RKLISCHMEKLISLDPSAFAPPURENT MVMIGMMLVLFRLQMAGASLPETELD DVCFQVELGFSTFLRSISSNSTVVNIKGTQIVIDQSIFGUHHEKLISLDFSAFAPPURENT MVRSAPSMEKNALINTVNTVMKAQSCVVQGS DVCFQVELGFSTFLRSISSNSTVVNIKGTQIVIDQSIFGUHHEKLSLFIFTNISHSKWMENNULLKIQDFAFANLFALRIYISEN MVRIGMEDFLGSVCQGGALNST RTFLGSKVQMKQCIPKNTTYVEIKLTGITVFFSKASSLDDKRTNVSENG MVUSMMLCFILGCSMANTEDTLASQFCAFNOST RSFVCLGNKVLDIPERTFTNTTFVEIKLTGISVFRAALSELMEKRIVVSENG MTPLWVLFLGLVSGTSCVAMHVCLANGTT RSFFCVGSRVQMPAVIPKNISYIEKLIGLRLIPRAFSELHEFSRIMSSENG	120 120 120 105 111 83 88 83
	Sa Dl Ea Ol Gm Tf Dr Aa	VALKNIGAFAFSEIPELTEITITKSEHLTHINPDAFKDIVKLKYLTIANTGLRVFPDFTKINSTG.VLLFDIHENSHIERVPANAFRGLCSQTITEIRITRNGIKEVASDAFNGTKMHRL VALKNIGAFAFSDIPELTEITITKSKHLTHINPDAFKDIVKLKYLTIANTGLREFPDFTKIHSTG.LLLFDIHENSHIERVPANAFRGLCTQTIPEIRLTRNGIKEVASDAFNGTKMHRL VALES GASAFSDIPELTEITITKSKHLTHINPDAFKDIVKLKYLTIANTGLREFPDFTKIHSTG.LLLFDIHENSHIERVPANAFRGLCTQTIPEIRLTRNGIKEVASDAFNGTKMHRL MALKKGAFAFSNIPALTEITITKSKHLSIHESAFRGLSELQVITISNTGLTFFNFSKINSAFSLDIQCN HIGLFANAFRGLCTQTIPEIRLTRNGIKEVASDAFNGTKMHRL VALES GASAFSDIPALTEITITKSKHLKSIHESAFRGLSELQVITISNTGLTVFPNFSKINSAFGLLVULENSFLQVVGNAFRGLCTVTIDEIQLTRNGTFEVASDAFNGTKMHRL VALES GASAFSNIPALTEITITSKHLKSIHESAFRGLSELQVITISNTGLEVPEPESRISTISGLEVULENSFLQVVGNAFRGLCTVTIDEIQLTRNGTFEVECCAFNGTKHRL VALES GASAFSNIPALTEITITSKHLKSIHESAFRGLSELQVITISNTGLEVPEPESRISTASGLEVULENSFLQVVGNAFRGLCTVTIDEIQLTRNGTFEVECCAFNGTKHRL ALERGEAAFANIKLEPITITSKHLVSHKARNUVRHENDERNTENGTISSTISSAFGLEVPEPESRISAAFGFLFDLQDNHIGVIFSNAFGGLSTGITELRITKNGTFEVECAFNGTKHRL ALERGEAAFSNISNIVETITTSKNUV.HKDAFNRIPERIRVITISNTGLKSLEPESKINSAALEFFLDLQDNHNGVIFPNAFLGISSETIFELRITKNGTFEVLNAFNGTKIGKI ALERGEAAHAFSNISNIVETITTSKNUVFIEKGAFNNISREKVITISNTGLKSLEPESKINSAALEFFLDLQDNNHVIHPNAFLGISSETIRELTIKNGTEVLNAAFNGTKLKL 1 1 af 1 leitk 1 f 1 lintgl p 5 l n naf gl t e lt ngi e afn t 1	239 293 240 225 231 202 206 202
	Sa Dl Ea Ol Gm Tf Dr Aa	FLGENÇQÎTHÎNPNAFVĞSSELVVLÜISHTALSSLEDYILGELÇKÎTAKSAFHÎKEL PPLQLESKÎRÇÂNLTYPSH FLGENÇQÎTHÎNPNAFVĞSSELVVLÜISÇTALSSLEDYILGELÇKÎTAKSAFHÎKEL PPLQTETELQANLTYSH FLGENÇQÎTHÎSPNAFVĞSSELVVLÜISÇTALSSLEDYILGELÇKÎFAÇSAFHÎKEL PPLQTETELQANLTYSHCCAF NIHRNESKWHSLCSHPLAÇGNLHFYRDYCSNSTSI FLGENÇQÎTHÎSPNAFVĞSSELVVLÜISTALSSLEDSVLGELÇKÎFAÇSAFHÎKEL PPLQTETELQAHLTYSHCCAF NIHRNESKWHSCSHPLAFEL SIKGNRÇÎTHÎSPNAFVĞSSELVVLÜISTALSSLEDSVLGELÇKÎFAÇSAFHÎKEL PPLQTETELQAHLTYSHCCAF NIHRNESKWHSTCSHPLAFT ILTENQLÎTHÎHSORAFVĞSSELVULÎSTALSSLEDISLEGELESAYHKÎ HPPQÇTAKÎRAKLTYSHCCAF KANFRESKWASUCSHPLAKTIDFYRDHCSNSTSI ILTENQLÎTHÎHSORAFVĞSAGLILDVSMTAVBSLESSLEELETLGENARÇÎRTIPLEGREHAMLTYHNECCAF LSM.SKKMESICSHPLAKTIDFYRDHCSNSTSI FLMENÇQÎKRÎHNYAFLQAEQTULDÎSKTAJSLEDINLERÎKLÎ JASVYSÎRWLPHLEIFTELÎÇANLTYSHCCAF KANKAKNKAKNEÇM.SIRMEPHFFKEHÇAVDIEV FLMENÇQÎKRÎHNYAFLQAEQTVLÎÎSKTAJSLESNLENLERÎKLÎ JASVYSÎRWLPHLEIFTELÎÇANLTYSHCCAF KANKKAKNAĞANNEGM.SIRMEEPHFFKEHÇADULEV LIMCNÇQÎRÇÎHSÇAFĞAEGPVVLÎDÎSKTAJSLESINLERÎKTIL BISVYTÎKKLENLELÎTELÎ TÇÂNITYSHCCAF KANKEKNKŞKNEÇM.VIGHEFDFENFENFENDEKDIEV LÎMCNÇQÎRÇÎHSÇAFĞAEGPVVLÎDÎSKTÎSVLEPINLKRÎKTÎ TESVYTÎKKLENLÊLIFTEÎ ÇÂNITYSHCAF KKEKKNENÇMÇM.VIGHEFDFENFENFENDEXDI LÎMCNÇQÎRÇÎHSÇAFĞAEGPVVLÎDÎSKTÎ PILÎFÎRÎKÎ TA BIYYTÎKKLENLÊLETELÎ ÇÎNTIYSHCAF KKEKREK KUNÇMÇM.VIGHEFDFENFENFENDE LÎMCNÇQÎRÇÎHSÇAFĞAEGPVVLÎDÎSKÎ DENLEHÎRÎ TALSYYTÎKKLENLÊLFTEÎ ÇÎNTIYSHCAF KKEKKEK KUNÇMÇM. VIGHEFDFENFENFENDÊ LÎMCNÇQÎRÇÎHSÇAFĞAEGPVVLÎDÎSKÎ DENLEHÎRÎ TALSYYTÎKKLENLÊLETETÊ ÇÎNTYRÎNCÊ KEKREK KUNÇMÊN. VIGHEFDÊ PÎNÊ TÊN TÊN	355 355 354 341 343 318 325 317
	Sa Dl Ea Ol Gm Tf Dr Aa	TCTFIQDEPNECEDINSEVPERVITUWILSILALIGNAVIAULGESRSKLTVPRFIM <mark>ELAISDIGUGIYAUVIATUDMI</mark> TRGRYNHAIDWGTGLGCS <mark>MAGITVFASIASVFILTAIT</mark> ICTFIQDEFNECEDINSAVPERVIJWIISILALIGNTAVILVIEGSRSKLTVPRFIMCHLAFSDLCMGIYLVVIATUDMITGGQYNHAIDWGTGLGCSAGEFTVFASELSVFILTAIT ICSFIEDARNECEDINSAVPERVIJWIISVLALIGNTAVILVIEGSRSKLTVPRFIMCHLAFADLCMGIYLIVIATUDMFTRGQYNHAIDWGTGLGCSAGEFTVFASELSVFILTAIT CSFIEDARNECEDINSAVPERVIJWIISVLALIGNTAVILVILGSSRSKLTVPRFIMCHLAFADLCMGIYLIITAITUMINEGYNHAIDWGTGLGCSAGEFTVFASELSVFILTAIT CSFIEDARNECEDINSAVPERVIJWIISVLALIGNTAVILVILGSSRSKLTVPRFIMCHLAFADLCMGIYLIVIATUDMFTRGQYNHAIDWGTGLGCSAGEFTVFASELSVFILTAIT CSFIEDARNECEDINSTFIRVIJWIISULALIGNTAVILVILSSRTKITVPRFIMCHLAFADLCMGIYLIVIATUDMFTRGQYNHAIDWGTGLGCSAGEFTVFASELSVFILTAIT SCYPRPAFNECEDINGFTFRVIJWISULAVIGNTVLIVILSSRTKITVPRFIMCHLAFADLCMGIYLINIASVUVIAMGDAVTRGQYNHAIAWGDYGGGAGCSAGEFTVFASELSVFILTAIT SCYPRPAFNECEDINGFTFRVIJWISULAVIGNFTVLIVILSSRTKITVPRFIMCHLAFADLCMGIYLILIASVDINGTGSYNYGIDWGTGGGAGGSAGEFTVFASELSVTILTAIT SCYPRPAFNECEDINGFTFRVIJWISULAIVGNTVLIVILSTRKKITVPRFIMCHLAFADLCMGIYLLILASVDINGTBYNYGIDWGTGGGGAGGFTVFASELSVTILTAIT SCHERPPAFNECEDINGFTYLRVIJWISULAIVGNTVLIVILSTRKKITVPRFIMCHLAFADLCMGIYLLILASVDINGTBYNYGIDWGTGGGGAGGFTVFASELSVTILTAIT SCHERPPAFNECEDINGFTYLRVIJWISULAIVGNTVLIVILSTRKKITVPRFIMCHLAFADLCMGIYLLILASVDINGTBYNYGIDWGTGGGGAGGFTVFASELSVTILTAIT SCHERPPAFNECEDINGFTYLRVIJWISULAIVGNTVLIVILSTRKKITVPRFIMCHLAFADLCMGIYLLILASVDINGTBYNYGIDWGTGGGGAGAFFTVFASELSVTILTAIT SCHERPPAFNECEDINGFTYLRVIJWISULATVGNTVLIVILSTNKITVPRFIMCHLAFADLCMGIYLLIASVDINGTBYNYGIDWGTGGGGAGAFFTVFASELSVTILTAIT C p d npc dim lrvl w sla gn vl vl s kltvprfimchlaf d cmg yl ia d t yn iwq gc agf tvf selsv tl tit	475 475 474 461 462 438 445 437
	Sa Dl Ea Ol Gm Tf Dr Aa	ERW HITHALRIDRKLEIRHACHWITAGNHESSIGAL PILGUSSYSKVSICLPMDVESLVSGVYV <mark>SIALANNAA SEWCOCVISINH</mark> IVRNESSAPAH.ADTRVAGRMAVIFTE ERW HITHALRIDRKLEIRHACIVWITAGNIESSIGAL PILGUSSYGKVSICLPMDVESLUSGVYVVSILLINIA FEVVGGCYLSINITVRNESSAPAH.ADTRVAGRMAVIFTE ERW HITHALRIDRKLEIRHACIVWITAGNIESSITALLPIVGISSYGKVSICLPMDVESUSGVYVVSILLINIA FEVVGGCYLSINIAVRNESSAPAH.ADTRVAGRMAVIFTEL ERW HITHALRIDRKLEIRHACVWAAGNIESSITALLPIVGISSYGKVSICLPMDVESUSGVYVVSILLINIA FEVVGGCYLSINIAVRNESSAPAH.ADTRVAGRMAVIFTEL ERW HITHALRIDRKLEIRHACVWAAGNVEALAAALPIIGVSSYKVSVCIPHDVESUSGVYVVSILLINIA FEVVGCYCYSINIAVRNESSAPAH.ADTRVAGRMAVIFTEL ERW HITHALRIDRKLEIRHRACVWAAGNVEALAAALPIIGVSSYKVSVCIPHDVESUSGVYVGLLILNIA FEVVGCYSINIAVRNESSAPAH.ADTRVAGRMAVIFTEL ERW HITHAMELERGLEIRHRACVWAAGNVEALAALPIIGVSSYKVSICIPHDVESUSGVYVGLLILNUAFHVVCYCYSINIAVRKESSTPAHAADTDVAGRMAVIFTEL ERW HITHAMCIERGLERHRACVWAAGNVEALAAALPVIGVSSYKKSICIPHDVETVSGQVYVLLILINUAFHAVCTYVKINITVRHEASVPES.ADARVAKMAVIFTIFL ERW HITHAMCIERGLERHRACVWAAGNVEALLAALPVIGVSSYKKSICIPHDVETVSGQVINLLILNAAFUVCYCYTINITVRHEAVENS ADARVAKMAVIFTIFL ERW HITHAMCIERGLERHACVWAAGNVEALAALPHIGVSSYKSICIPHDVETIVSGQVINLLILNAAFUVCYTINITVRHEASVES.ADARVAKMAVIFTIFL ERW HITHAMCIERGLERHACVANAGNIFTISCIPHTENGSSYKSICIPHDVETILSQAYINLLIANAFUNGSYVSINIA ADN HARMAVIFTIFL ERW HITHAMCIERGLERHACVANAGNIFTISCIPHTENGSSYKSICIPHDVETILSQAYINLLIANAFUNGSYVSININAAFNAVIFTIFL ERW HITHAMCIERGURFRACVANAGNIFTISCIPHTENGSYSYKSICIPHTISGAVIITILANAFNAVIFTIFL ERW HITHANCHERGURFRACVANAGNIFTISCIPHTENGSYNKISTICHTISGAVITILIANAFNAVIFTIFL ERW HITHANCHERACVANAGNIFTISCIPHTENGISSYKSICIPHTISGAVITITINAAFNAVIFTIFL ERW HITHANCHARACVANAGNIFTISGISSYKSICHTENGTITISGAVITITINAAFNAVIFTIFL ERW HITHANCHARACVANAGNIFTISGISSINAALLENAAFNAVIFTIFL ERW HITHALRACVANAGNIFTISGISSINAALLENAATUFTIFL	594 593 580 582 557 564 556
	Sa Dl Ea Ol Gm Tf Dr Aa	MADIGIFATGAMIKRPLITISD <mark>KKMAUNIVIPUNGGENEINY HET</mark> RETERDEFLIAARFGLEKTRAGIYRTESSSCQQPAWT.SPRGGHVMLVSLANTLSVDGKHE CMAPISFFAISAALKLPLITVSDAKLLUVLFYPINSCAMPELYAFTRETERDEFLIAARFGLEKTRAGIYRTESSSCQQPAWT.SPRSGHVMLVSLANALSLEGKPE CMAPISFFAISAALKLPLITVSDAKLLUVLFYPINSCAMPELYAFTRETERDEFFLIAARFGLEKTRAGIYRTETSSCQQPAWT.SPRSGHVMLVSLANTLSUDGKQD GMAPUSFFAISAALKLPLITVSDAKLLUVLFYPINSCAMPELYAFFTRETERDEVFLAARFGLEKTRAGIYRTETSSCQQPAWT.SPRSGHVMLVSLANTLSUDGKQD CMAPISFFAISAALKLPLITVSDAKLLUVLFYPINSCAMPELYAFFTRETERDEVFLAARFGLEKTRAGIYRTETSSCQQPAWT.SPRSGHVMLVGUNT CMAPISFFAISAALKLPLITVSDAKLLUFYPINSCAMPELYAFFTRETERDEVFLAARFGLEKTRAGIYRTETSSCQQPAWT.SPRSGHVMLVGUNT CMAPISFFAISAALKLPLITVSDAKLUVLFYPINSCAMPELYAFFTKREVLAARFGLEKAAQGIYRTETSSLHNGPRCSARNSDGILVSLGHVAHPH CMAPISFFAISAALRQPLITVSHAKVLUVLFYPINSCAMPELYAFFTKREVLAARFGLEKRAATIYCTEISSGQNGAVVFSHTSDGILYSLVHIAQVH CMAPISFFAISAALKUPLITVSHAKVLUVLFYPINSCAMPELYAFFTKREVENFIASHGCEKRAATIYCTEISSGQNGAVVFSHTSDGILYSLVHIAQVH TMAPISFFAISAALKPLUTVSHAKVLUVLFYPINSCAMPELYFFTKREKEDFFIASHGCEKRAATIYCTEISSGQNGAVVFSHTSDGILYSLVHIAQVH TMAPISFFAISAALKPLUTVSHAKVLUFYPINSCAMPELYFFTKREKENFINSTERGEVERAATIYCTEISSGQNGAVVFSHTSDGILYSLVHIAQVH TMAPISFFAISAALKPLUTVSHKAKVLUFYPINSCAMPELYFTKEKKENFINGTENSTCQCFTRATENEISSTCQCFTANELYSLVHIAQVH TMAPISFFAISAALKPLUTVSHKAVLUFYPINSCAMPELYFTKEKKENFINSTERGEVERAATIYCTEISSGQNGAVVFFKTSDIJSLVHIAQVH TMAPISFFAISAALKPLUTVSHKAVLUFYPINSCAMPELYFTKEKKENFINSTENGANGAVGNAVGNAVFFKTSTISTAVINTEN TMAPISFFAISAALKANVINTSTENSTANTAGUNT	701 700 686 683 661 668 660
Aa Dr Ea Gm Ol Tf Sa Con	sensu	MTPLWVLFLGLVSGTSCVAMHVCLANGTTRSFFCVGSRVNQMPAVIPKNTSYIEFKLÖQLRLIPRAAFSEÜHELSRIMVSENG MVLSMMLCFILGCSMANTEDTLAASQFCAFNGSTRSFVCLGNKVLEIPRRIPTNTTFVEIKLÖQISVFRRAALSEÜHELKRIVVSENG MUMIMILIMLMTVMIKTAAASAPGPEMDIKPCATNGLAKKTLHFCYQLGYGVTEIPSNISSDTQCLEVQQQQIKVIPQCALTSLQHUKKLIISK DMLESITASAFAGLPQLTEIFISEY MRESAPSMRKMALMTNTVMTVMKGAQSCVVQGSGTVECVGGNITEMPSLSPHTTVTLNISQTHIKGLSLETFTNLSHUSKVWIMKLVUMLLKIQDFAFANLPALPALRIIYISEN .MVVMIQMMLVLFRLQMAGASLPETELDDVCFQVELGFSTFLRSISSNSTVVNIKQTQITVIDQSIFTGUWHDEKLTILDNDKLLSICPSAFANLPRLFDVSIXQN MLRFTLSWLMMHAGDMFLGSYACLANGTTRTFLCLGSKVHQMPCQIPKNTTYVEIKLTQIIMFPSKAMSSUHDUKRIMVSENG MMMVMILIMFMMLMIKTATASVPGPEMHVKPGVETSLAIRTLSFSYQLKFGVTEIASSIPSNTCLVVKQTEIVVIQQALNSLQHURKVTIWENDKLESINEFAFASLSQLTDFFISGN S t 1 1 n	83 88 120 111 105 83 120
Aa Dr Ea Gm Ol Tf Sa Con	sensu	. ADEGTAAHAFSNISNIVEITITKSKNIVFIEKGAFWNLSRUKYLTISNTGLKSLIDFSKINSAAKDFLFDLQDNYMKVIHPNAFLGLSSETIRGLRUTKNGITEVLNHAFNGTKLDRL . ADEGTAAHAFSNISNIVEITITKSKNIVFIEKGAFWNLSRUKYLTTISNTGLKILDFSKINSAALEFLFDLQDNHTERIPSNAFLGLTNATITDLRUTKNGTREVISHAFNGTKLGKL VALESTGASAFSDLPELTETTTKSKNIVT MHKDAFWRLMRURYLTISNTGLTTFNFSKINSAALEFLFDLQDNHTERIPSNAFLGLTNATITDLRUTKNGTREVASDAFNGTKTGKL PATETTGSNAFSNISAL ETTTKSKNIVT MHKDAFWRLMRURYLTISNTGLEVEPTERSTILTTSGELLULDEN SFLGRYPONAFRGLCTOTTDLRUTKNGTREVASDAFNGTKMHRL PATETTGSNAFSNISAL ETTTKSKNIVT MSSAFRGLSELOYLTISNTGLEVEPTERSTILTTSGELLULDEN SFLGRYPONAFRGLCTOTTDLRUTKNGTREVASDAFNGTKMHRL NAUKKTGAFAFSNIPALTETETTKSKNIVT MSSAFRGLSELOYLTISNTGLEVEPTERSTILTTSGELLULDEN SFLGRYPONAFRGLCTOTTDLRUTKNGTREVASDAFNGTKMHRL . VDQSTEAYAFANJTKLEETTTKSKNIVT MNTTFWGLPRURYLTISNTGLTVLDFSNAFSVAGAFFFLFDLEDNHTQVPSNAFRGLCTOTTELLTKNGTTEVOTAFNGTKEKK VAQSTEAYAFANJTKLEETTTKSKNIVT MDTFWGLPRURYLTISNTGLTVLDFSNAFSTLFDLSKTHSTGFLFDLENTTGLTSNAFRGLCSQUTTGLNGTKMRT . VDQSTEAYAFANJTKJEETTTTKSKNIVT MDTFWGLPRURYLTISNTGLTVLDFSNAFSTCUTDTGLTFTGLSTAFTSTLFTNGTKEVASDAFNGTKMHRL . VDQSTEAYAFANJTKJEETTTTKSKNIVT MOTTFWGLPRURYLTTSTGLTVLDFSNAFGTGLTGLCSQUTTGLTTKSKNIVT MATTGLTSTGFLFTTTTSKSAFFGTGTTGLSQUTTGLSTAFTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT	202 206 240 231 225 202 239
Aa Dr Ea Gm Ol Tf Sa Con	sensu	LLMGNQQLRQTHSQAFSGAEGPVVLDVSRTSISVLPENILWRUKRUTMESVYTUKKLPNLDLTTQTIEANLTYRSICCAFANSKKNMSVVHELQDKPNIKQEEPQWHLEHCTIEV FLMGNQQLNHHSYAFKGAEGPVVLDVSRTSISVLPENILWRUKRUTMESVYTUKKLPNLDLTTQTIEANLTYRSICCAFANSKKNMSVVHELQDKPNIKQEEPQWHLEHCTIEV FLMGNQQLNHHSYNFKGAEGPVVLDISETALSSLPDSVLGGLQKUFAQSAFHLKELPPLQLGTEURQAHLTYPSICCAFKNFKKHKSVKNQMON.VTGAHEEPDFNFFRDHCKDVIEV FLMGNQLTHISPNAFVGSSELVVLDISETALSSLPDSVLGGLQKUFAQSAFHLKELPPLQLGTEURQAHLTYPSICCAFANSKKNMSVVHELQDKPNLV.WFFQAHCFNFTSI IDTGNHQLTTHPNAFSGSAGLTLLDVSNTAVRSLPESILEGUETUGGENARQLRTLPP.LEGRUKHANLTYHWICCAFLDSN.SKSKWDSIGSQPEAHLPIY.WKFQAHCFNFTSI SLKGNKQLTHISGDAFVGSSELVVLDISETAISSLPDNIISCUKKULDESAVHUKLLPPQQGAKURLAKLTYPSICCAFKNKPRSRSKWSPLGSHPMAKYITDFYRDHCSNSTSI FLUGNQQLKKNHNYFFLAAEGPLVLDISETAISSLPENURRKKUUHTSVSNERULPPLQLGTEITEITONNITYPSICCAFKNFKKKKAKNHFON.DSIRKEPD.HFFKEUCKOVIEV FLUGNQQLTHINPNAFVGSSELVVLDISHAISSLPDVILGGUGKULHKSAFHLKELPPLQLGSKHOANLTYPSICCAFQNIHRNRSRWSSLQSHPKAPDNLHFYRDHCSNSTSI SI gn ql i af g ld st lp l l a l lp f l a lty hcc s c hc	317 325 354 343 341 318 355

⁽图 3 Fig. 3)

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Aa S Dr Ea G Gm Ol Tf Sa Consensus	SCHPKPDAF TCYPTPDAF TCSPIPDAF CSPIPDAF CSPTPDNI SCYPKPDAF TCTPTQDDF CPd	NPCEDII NPCEDII NPCEDII NPCEDII NPCEDII NPCEDII NPCEDII NPCEDII npc di	NGFTYL NGFTFL NETSPL NETSPL NGFTFL NGFTFL NSPVPL m 1	RVLIW RVLIW RVLIW RVLIW RVLIW RVLIW RVLIW rVI W	IISVL FISVL IISVL VISLL IISVL FISVL IVSIL s 1	AIVGNS AIVGNT ALLGNT ALLGNA ALLGNT AVLGNF AVLGNF ALLGNA a gn	VVLLVL VVLLVL AVLLVL TVLLVI VVLVVI TVLLVI AVLLVL v1 v1	ITSHY LTSRY FGSRS LASRA LGSRS LSSRT LGSRS S	CLTVPRF CLTVPRF CLTVPRF CLTVPRF CLTVPRF CLTVPRF CLTVPRF Altvprf	FLMCHLA FLMCHLA FLMCHLA FLMCHLA FLMCHLA FLMCHLA FLMCHLA FLMCHLA	FADLC FADLC FSDLC FADLC FADLC FADFC FSDLC f d c	MGVYLF MGIYLL MGIYLV MGVYLV MGIYLI MGLYLL MGIYLV mg yl	VIACVD LIAAVD VIATVD VIATUD IIATID IIASVD VIATVD ia d	IHTKSR IHTQSR MFTRGQ MVTRGQ MLTHGH VQTRSH MLTRGR t	YNYGI IYNYGI HNHAI IFNHAI IYNYAI IYNYGI IYNHAI 7 n i	DWQTG DWQTG DWQMG AWQMD DWQTG EWQTG DWQTG WQ	PGCGAA AGCHVA LGCSAA YGCNIA LGCSAA AGCSSA LGCSAA gc a	GFFTVFA GFFTVFA GFFTVFA GFFTVFA GFLTVFA GFLTVFA GFFTVFA gf tvf	SELSVY SELSVF SELSVF SELSVF SELSVF SELSVF SELSVF SELSVF selsv	TLTMIT TLTAIT TLTAIT TLTAIT TLTAIT TLTAIT TLTAIT TLTAIT tlt it	437 445 474 462 461 438 475
Aa Dr Ea Gm Gm Gm Gn Sa Consensus	LERWHTITF LERWHTITY LERWHTITY LERWHTITY VERWHTITY LERWHTITY LERWHTITY erwhtit	AMQLDR AMQLER ALRLDR ALRLDR ALRLDR AMRLER AMRLER ALRLDR a 1 r	KFRLRH QMRLRH KIRLRH KIRLRH KIRMRH QLRLHH KLRLRH r h	ACAVM ACLVM ACIVM ACVVM ACVIM ACGVM ACGVM ACIVM ac m	AG <mark>GWL</mark> ATGWL TAGWI AAGWV AAGWI ALGWL TAGWI gw	FASMAA FSLLAA FSSLTA FALLAA FSSLAA FSVLAA FSSLSA f a	LLPTAG LMPMFG LLPTVG ALPTLG LLPTVG LMPVIG LLPTLG P S	VSSYM VSSYS ISSYG VSSYD VSSYS VSSYM VSSYS SSY	WSICLF TSICLF WSICLF WSICLF WSVCLF TSICLF WSICLF Sclf	MDVETP MDVETL MDVESV MDVDSL MDVESL MDVETV MDVESL MDVESL	VSQAY LSQGY VSQVY LSQVY VSQVY SSQVY SQVY SQVY SQVY	IIILL VVLLLL VVSLLL VVGLLL VVGLLL VNLLLI VVLLLI 11	FNVLAF LNALAF LNILAF LNVLAF LNILAF LNVLAF LNVLAF LNILAF n af	LVICVC LVVCVC VCVCGC LVVCVC FCVCGC MAVCTC FCVCCC c c	ILSTYL TLTYL ILSTYL FSTYL ILSTYL VRTYL ILSTYL ILSTYL I	TVRNP TVRNP AVRNP TYRKP TVRHP TVRNP r p	SFVSAN AFVPAN SSAPPH SSTPAH SSAPAH ASVPDS SSAPAH	ADMRIA ADMRIA ADTRVA ADTRVA ADTRVA ADTRVA ADARVA ADRVA ad a	KRMAVL KRMAVL QRMAVL QRMAVL QRMAVL KRMAVL QRMAVL QRMAVL rma 1	IFTDFL IFTDFL IFTDLV IFTDFL IFTDFL VFTDFL IFTDFL IFTDFL	556 564 593 582 580 557 594
Aa (Dr (Ea (Gm (Ol (Sa (Consensus	CMAPISFFA CMAPISFFA CMAPISFFA CMAPISFFA GMAPUSFFA CMAPISFFA MAPISFFA MAPISFFA	ISAALK ISAAFK VSAALK ISAALK ISAALK ISAALK ISAALK ISAALK ISAALK	LPLITV LPLITA LPLITV LPLITM LPLITV QPLITV RPLITI plit	SHSKV Shakv Sdakl Sdakl Shakv Sdakl k	LLVLF LLVLF LLVLF LLVLF LLVLF LLVLF LLVLF 11vlf	YPINSC YPINSC YPINSC YPINSC YPINSC YPINSC YPINSC YPINSC ypins	SNPFLY SNPFLY ANPFLY ANPFLY SNPFLY ANPFLY SNPFLY npfly	AFFTKI AFFTRI AFFTRI AFFTRI AFFTRI AFFTRI AFFTRI AFFTRI afft	IFKRDFF IFRRDFF IFRRDFF IFRRDFF IFRRDFF IFRRDFF IFRRDFF IFRRDFF	FILASHF FILTSRF FFLTARF FLLAARF FLLAARF FLLTSRF FLLAARF 1 f	GCFKT GCFKR GLFKT GLFKA GLFKT GCFES GLFKT g f	QAQIYR RAHIYC RAQIYR QAQIYR RAQIYR HARVYR RAQIYR RAQIYR A y	TEISSV TEISSG TETSSC TESSTC TETSSC TETSSI TESSSC tess	QNGAWVI QQQAWT. QQPAWT. QQPAWA. QQPTWT. HNGPRMO QQPAWT.	PTPKTS SPKTS SPKSS SPKSS SPKSS CSAKNS SPKGS k s	DGTLY DGTLY HVMLY HGTLY RVM.Y DGTLY HVMLY y	SLVPIN SLVHIA PVTNTS SLANIS SLANTL SLGHVA SLANTL	NPH QVH SQDGKHN H SLDGKQE HPH SVDGKHE			660 668 700 683 686 661 701

图 3 金钱鱼FSHR氨基酸序列与其他物种FSHR氨基酸对比

7次跨膜螺旋区, CCAF、ERW、FTD、NPFLY: GpHR家族的特殊保守信号序列。GenBank登录号: KU646840(金钱鱼.Sa), AAV48628.1(欧洲鲈.Dl), AIW52567.1(点带石斑鱼.Ea), BAJ05402.1(青鳉.Ol), ABD62885.1(大西洋鳕.Gm), AEH84420.1(黄颡鱼.Tf), AAR84280.1(斑马鱼.Dr), CPR30242.1(欧洲鳗鲡.Aa)

Fig. 3 Alignment of FSHR from several species

Seven transmembrane helix domains, CCAF, ERW, FTD, NPFLY: special conversed signal sequence of GpHR family. GenBank accession number: KU646840(*Scatophagus argus*. Sa), AAV48628.1(*Dicentrarchus labrax*. Dl), AIW52567.1(*Epinephelus akaara*.Ea), BAJ05402.1(*Oryzias latipes*. Ol), ABD62885.1(*Gadus morhua*. Gm), AEH84420.1(*Tachysurus fulvidraco*. Tf), AAR84280.1(*Danio rerio*. Dr), CPR30242.1(*Anguilla anguilla*. Aa)

Aa Dr Dl Gm Ol Sa Tf Consensus		89 78 86 87 90 119 90
Aa Dr Dl Gm Ol Sa Tf Consensus	SASLEI LEALAINNI TNISE LI JONTRUAH ISKAARNNIPKLOVISISYIGISUPPLSA ISSLOSNFILDICOVINUSI RSVARIGHT. KEHTOMNIYNGFKEVODHAFNGTRINK SSSVETIESEAINNIPYNSE ISI QATRA VHIQQRARAQUPKLWYUSISYIGISY PPLTSIFSLEAHFILDICOVINURSVESAAFTANI. SEVATAN IPNGFQEIESHAFNGTRINK SVTLETIEALAINNINISE ISVQATRS MHIGRRTRANLPKLHYUSISYIGISY PPLTSIFSLEAHFILDICOVINURSVESAAFTANI. SEVATAN IPNGFQEIESHAFNGTRIDK SVSVELEQRAALSJALCSE ISIQATRS MHIGRRTRANLPKLHYUSISYIGITY PPLTSIFSLEAHFILDICOVINURSVESAAFTANI. SEVATAN IPNGFQEIESHAFNGTRIDK SVSVELQRAALSJALCSE ISIQATIS RATUKKAAFTANIPKINYUSISYIGITY PPLTSIFSLEAHFILDICOVINURSVESAAFTANI. SEVATAN IPNGFQEIESHAFNGTRIDK SVSVELQRAALSJALASHASIN SARI BERGARTANLPKURYUSISYIGINH PPLTSISSLAMSTIIEWADATKIDSIAASSARAFA. EDYIFMA VARGFKEIHSHAFNGTRIDS SDRITALQRHAFLSHSLQQULVLNINS RGIERGARTALPREFTISYIGINH PPLTSISSLAMSTILEWADATKIDSIAASSARAFA. SCHTENTAN VARGFKEIHSHAFNGTRINS NAVLIKUGAAFSE PPETLETITISSTITHMPDARADIVKVUNUTININGFKEIHSPARAFA. SULEMULANATAN SCHTESISSAAFTANI. SAAFTANIS SDRITALQRHAFLSHISLIGUTUSISTITISSEN TAUKUN INTOKINA VARGFKEIKSAAFTANISTISSAAFTANISTISSAAFTANISTISSAAFTANISTAN SAAFTANISTISSAAFTANISTISSAAFTANISTISSAAFTANISTISSAAFTANISTANISTAN SAAFTANISTISSAAFTANISTANISTISSAAFTANISTANISTISSAAFTANISTISSAAFTANISTANISTANISTANISTANISTANISTANISTANIS	208 197 205 206 209 238 209
Aa Dr Dl Gm Ol Sa Tf Consensus	V KNNKK KNIHSDAFKGAFGPST DV SATAVEAL DERGUEAVLV V RGAYALKSDP LTGLQSDQE QU TYPSTICGSLREADVFAEWS QRSAFCNDVSLLERMLALS VV KNNKD RVVHEDAFKGALGPTVDVSSTALETLESHGJESVLMITTRSAFALKK DPDKSLKSTRE VOLFPSTICGALINW. DNSRDGSVNSALR RSSYCGDNSSPADLSAIS VV KNNKN RVIHKYHEGAFGGVDDVSTALTKLPQGJESVLMITTRSAFALKK DPDKSLKSTRE VOLFPSTICGALINW. DNSRDGSVNSALR RSSYCGDNSSPADLSAIS UV KNNKN RVIHKYHEGAFGLEAVVDISSTALETLESHGJESVLMITTRSAFALKK DPDKSLKSTRE VOLTYPSTICGALSW. NTRIPSINPAYNDSTYCDESDQLARVQRVIGGS DDRONKH VNHEGAFDGLEAVVDISSTALSEPAGETVVFFQGJESVLVFFQQSAYALKSDPDAGLWSLEHHUTYPSTICGAFG RKARFSALKKLTRLEVADAFADDG LI RDNRFLSYIAEDAFEEATGPSUDVSSTALSALEAKGITRVQTIK TATFALKSDPDASLEELEVELTYPSTICGAFGRKARFSALKKLTRLEVELTOVEADMEPADDG FFGGAQQITHINPNAFVGSSELVVDII NITALSSLDVROITK TATFALKSDPDALFSNHQAVITYPSTICGAFQ	318 313 324 316 322 339 326
Aa Dr Dl Gm Ol Sa Tf Consensus	VDLPLAPESVDPSPEVYSSIDLHYPEFDF, GISRRAPRGADEADAFNPCEDIVGFGFLRVAIUVISLAIVGNLTVIAVLVASRRKINVPFGFUNGHLAFADLGIGVUULTTAAV SDDSLESDVIGSSSVEDTFGSIDFHYPDLDL, GQRQALQS BEADAFNPCEDIAGFSFLRVAIUFINILAIAGNLVVLVLVFSRCKLTVPRFUNGHLAFADLGIGVULUTTAAV ADTLVNDMPFFSDVDLSEDEGFG0VSHTYPELDF, GYTRPILVTUEADARPCEDIAGFSFLRVAIUFINILAIAGNLVVLVLVFSRCKLTVPRFUNGHLAFADLGIGVULUTATV 	431 428 443 417 423 432 426
Aa Dr Dl Gm Ol Sa Tf Consensus	DLRTRGDÜSRHALANOTG BEGGANGELSVEGGELSVYELTVVL BRUHTITNULRRDRK GLARAAVYMAT GULLCLSVALUPLLSVSSYGKVSVCLPVDIDTPCA TFVVLLULLVA DLRTRGHVSHHALBUQTGACCDIAGFLSVEGGELSVYELTVIL BRUHTITNALLLERRVGLSQASLIMTIGULLCLSVALUPLLGVSSYSKVSUCLPVDIETPLSAAVVILLULVAA DFRTGGHVSGHALBUVTGSGCSAAGFLSVEGGELSVYELSTILURRHTTINALGIERH HVLTGAASIMAAGUICLGWGMUPUFFVSSTAKVSUCLPVDIETPLSAAFVILL SYSSPOYNHAVDUGGGCSIAGFLTVASSELSVYELTVISLBRUHTITNAHLHKRVRLRHVATAMMAAGUFSLVALUPLVSSYSKVSICLPVDIETGSAFVVAL DFRSREMTNAHTDVGTGCGCAAGFLTVASSELSVYFUTISLBRUHTITNAHLHKRVRLRHVATAMMAAGUFSLVALUPLVSSYSKVSICLPVDIETGSSPVVAL DHLTRGNYNHAIDUGTGLGCSAAGFFTVESSELSVYFUTISLBRUHTITNAHLHKRVRLRHUHTVMAAGUFSLVALUPLVSSYSKVSICLPUDITSSOVVVTFULLVAA DALTRGNYNHAIDUGTGLGCSAAGFFTVESSELSVYFUTISLBRUHTITHAURARKRURLANGVSELVALUPLVSSYSKVSICLPUDITSSSVVVTFULLVAA DALTRGNYNHAIDUGTGLGCGAAFLTVESSELSVFTUTISLBRUHTITHAURARKRURLRHAVSTAMMAAGUFSLSALUPULSSSVSKVSICLPUDITSSSVVVTFULLVAA DALTRGNYNHAIDUGTGLGCGVAFFTVESSELSVFTUTIST	551 548 563 537 543 552 546 2.4)



图 4 金钱鱼LHR氨基酸序列与其他物种LHR氨基酸对比

7次跨膜螺旋区, ERW、FTD、NPFLY: GpHR家族的特殊保守信号序列。GenBank登录号: KU646840(金钱鱼.Sa), ABX79919.1(欧洲 鲈.Dl), NP_001117798.1(虹鳟.Om), AAR84281.1(斑马鱼.Dr), CPR30244.1(欧洲鳗鲡.Aa), ABD62886.1(大西洋鳕.Gm), BAJ05403.1(青 鳉.Ol), AEH16748.2(黄颡鱼.Tf)

Fig. 4 Alignment of LHR from several species

seven transmembrane helix domains, ERW, FTD, NPFLY:special conversed signal sequence of GpHR family. GenBank accession number: KU886226(*Scatophagus argus*. Sa), ABX79919.1(*Dicentrarchus labrax*. Dl), NP_001117798.1(*Oncorhynchus mykiss*. Om), AAR84281.1(*Danio rerio*. Dr), CPR30244.1(*Anguilla anguilla*. Aa), ABD62886.1(*Gadus morhua*. Gm), BAJ05403.1(*Oryzias latipes*. Ol), AEH16748.2(*Tachysurus fulvidraco*. Tf)



图 5 金钱鱼FSHR与其他脊椎动物FSHR构建的系统进化树

GenBank登录号: 欧洲鲈(AAV48628.1),点带石斑鱼(AEG65826.1),青鳉(BAJ05402.1),底鳉(BAF48336.1),大西洋鳕(ABD62885.1),安 大略鲑(ABH10576.1),虹鳟(AF439405.1),斑马鱼(AAR84280.1),黄颡鱼(AEH84420.1),尖齿胡鯰(CAB51907.2),欧洲鳗鲡 (CPR30242.1),原鸡(NP_990410.1),美洲洞蛇(AAO72730.1),家猫(NP_001041479.1),马(NP_001157485.1),小鼠(NP_038551.3),大鼠 (NP_954707.1),人(AAB26480.1),矮黑猩猩(XP_003822740.1)

Fig. 5 Phylogenetic tree of Scatophagus argus FSHR and other species FSHR

GenBank accession number: Dicentrarchus labrax (AAV48628.1), Epinephelus coioides (AEG65826.1), Oryzias latipes (BAJ05402.1), Fundulus heteroclitus (BAF48336.1), Gadus morhua (ABD62885.1), Salmo salar (ABH10576.1), Oncorhynchus mykiss (AF439405.1), Danio rerio (AAR84280.1), Tachysurus fulvidraco (AEH84420.1), Clarias gariepinus (CAB51907.2), Anguilla anguilla (CPR30242.1), Gallus gallus (NP_990410.1), Bothrops jararaca (AAO72730.1), Felis catus (NP_001041479.1), Equus caballus (NP_001157485.1), Mus musculus (NP_038551.3), Rattus norvegicus (NP_954707.1), Homo sapiens (AAB26480.1), Pan paniscus (XP_003822740.1)



图 6 金钱鱼LHR与其他脊椎动物LHR构建的系统进化树

GenBank登录号: 点带石斑鱼(AEG65827.1), 欧洲鲈(ABX79919.1), 底鳉(BAF48337.1), 安大略鲑(ABH10577.1), 虹鳟 (NP_001117798.1), 斑马鱼(AAR84281.1), 草鱼(ABM73668.1), 欧洲鳗鲡(CPR30244.1), 原鸡(BAA23736.1), 小鼠(AAA39432.1), 大鼠 (AAA41529.1), 人(AAB19917.2), 牛(AAM09535.1)

Fig. 6 Phylogenetic tree of Scatophagus argus LHR and other species LHR

GenBank accession number: Epinephelus coioides (AEG65827.1), Dicentrarchus labrax (ABX79919.1), Fundulus heteroclitus (BAF48337.1), Salmo salar (ABH10577.1), Oncorhynchus mykiss (NP_001117798.1), Danio rerio (AAR84281.1), Ctenopharyngodon idella (ABM73668.1), Anguilla anguilla (CPR30244.1), Gallus gallus (BAA23736.1), Mus musculus (AAA39432.1), Rattus norvegicus (AAA41529.1), Homo sapiens (AAB19917.2), Bos taurus (AAM09535.1)



图 7 金钱鱼卵巢、精巢不同发育时期形态结构

Fig. 7 Morphological structure of ovary and testis of S. argus at different development stages

1, 2, 3, 4 are the morphological structure of ovary at the development stages of I, II, III, IV, respectively.5, 6, 7, 8 are the morphological structure of testis at the development stages of I, II, III, IV, respectively. Oo. oogonia, Oc. oocyte, Gc. granulose cells, YG. yolk granules, Sc. sertoli cell, LL. primary spermatocyte, ST. spermatid, SZ. spermatozoa. Scale bar=50 µm



图 8 金钱鱼卵巢、精巢不同发育时期FSHR和LHR基因mRNA的相对表达分析 不同字母表示差异显著(P<0.05)



Bars with different letters are significantly different (P<0.05)

7期

这些都充分证明克隆所得到的序列是金钱鱼 GtRHs目的基因。金钱鱼性腺发育可分为4个时 期,不同发育阶段雌、雄的GtHRs的mRNA表达 水平不同。金钱鱼卵巢中FSHR在 I 期表达水平 较高,随着卵巢发育,表达水平显著下降。在 许氏平鲉(Sebastes schlegelii)卵巢中, FSHR具有 相似的表达方式,在卵巢的发育早期显著高于后 期^{16]}。在卵黄生成的早期,尼罗罗非鱼(Oreochromis niloticus)FSHR基因也有比较高水平的表达[17]。推 测金钱鱼FSHR在卵巢的早期发育中起着重要的 作用。不同的鱼类, 在卵巢发育早期FSHR的表 达水平也不相同。在斑马鱼中, FSHR表达水平 随着卵黄的生成而逐渐升高,在卵黄生成的中 期达到最高水平,随后开始下降[6]。在罗非鱼 中,FSHR在整个生殖周期的表达水平都较低, 但在排卵后开始逐渐升高^[17]。同样在红点鲑(O. rhodurus)^[18]、半滑舌鳎(Cynoglossus semilaevis)^[15]中,FSHR表达量都是在卵黄生成早期开始 上调,并在整个卵黄生成过程中持续上升,最 后在卵黄生成后期达到最高。以上结果表明 FSHR在鱼类卵巢的表达方式主要有两种, 金钱 鱼的FSHR属于在卵巢发育前期有较高表达水 平,出现两种表达方式的原因可能是由于鱼类 的生长易受外界因素影响,不同鱼类进化出不 同的表达方式。在雄性大西洋鲑中, FSHR的表 达水平在早期发育阶段明显高于后期¹⁹。且金钱 鱼FSHR在Ⅲ期精巢中的表达量明显高于在Ⅳ期 精巢中的表达量,这表明FSHR在精巢发育和精 子发生阶段起重要作用。金钱鱼LHR基因mRNA 在卵巢中Ⅰ、Ⅱ、Ⅲ期表达水平较低,且在 I、Ⅱ、Ⅲ期之间没有明显变化(P>0.05),而在 Ⅳ期时, LHR基因mRNA表达水平均有显著的升 高(P<0.05)。在雌性斑马鱼排卵期,颗粒细胞中 充满卵黄颗粒,同时LHR的表达水平达到最高⁶⁶。 金钱鱼LHR在卵巢中的表达方式印证了上述研究 结果。在雄性金钱鱼中,LHR的表达随着精巢的 发育逐渐升高,在IV期达到最高。在真鲷中, LHR主要调控排卵和排精^[19],雄性金钱鱼LHR的 表达方式与之相符。Rt-PCR结果显示了不同时 期GtHRs的表达水平,揭示了金钱鱼FSHR主要与 早期内源性卵黄的生成和精子的发生有关, LHR的主要作用是促进配子成熟和排出。本研究 首次克隆得到金钱鱼FSHR和LHR基因cDNA序列 全长,并对其在精巢和卵巢不同发育时期中的 表达进行了分析,初步揭示了FSHR和LHR在金 钱鱼生殖周期中的作用,为进一步研究硬骨鱼 类的繁殖及生理作用奠定基础。GtHRs或GtHs (FSH和LH)在金钱鱼性腺生长发育过程中的具体 机制还需进一步探究。

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Gene cloning of gonadotropic hormone receptors and their mRNA expressions at different development stages of the gonad in *Scatophagus argus*

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Abstract: In the present study, follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR) genes were cloned by using reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA 5' and 3' ends (RACE) in *Scatophagus argus*. The full-length of FSHR cDNA was 2538 bp, encoding a 702 amino acid protein and the full-length of LHR cDNA was 3315 bp, encoding a 722 amino acid protein. Both of them contain seven TM helix domains, a feature of glycoprotein hormone receptor (GHRs) family. Multiple sequence alignment shows that *S. argus* GtHRs have the highest homology with that of *Dicentrarchus labrax*. mRNA expression patterns showed that a high expression level of FSHR was detected at stage I in ovary, followed by an obvious decrease at stage II, III and IV. In the testis, the expression of FSHR gene increased gradually during early stages (stage I, II and III), and apparently reduced at stage IV. Our results indicates that according to H.E staining, sperm maturation started at stage III, implying FSHR played an important role in early stages of the gonadal development, and LHR is vital for sperms/oocytes maturation and release as well as sperms.

Key words: Scatophagus argus; FSHR; LHR; sequence analysis; gene expression

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