CLONING AND EXPRESSION OF KIT LIGAND AND ITS RECEPTORS IN THE EUROPEAN SEABASS

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Introduction

The Kit system consist of the growth factor kit ligand (or Stem Cell Factor) and its receptor c-kit. In mammals, among other functions, this system acts as regulator in several reproductive functions, especially during the early stages of gonadal development. The objective of the present work is to clone kit ligand (kitlg) and c-kit (kit) as a first step to study their involvement in the control of the initial stages of gonadal development in both males and females of European seabass, Dicentrarchus labrax, in order to increase the knowledge on the control of early stages of gametogenesis in fish.

Methods

Blast analysis against the NCBI whole-genome shotgun contigs (wgs) database for D. labrax (sequences CBXY010000001 – CBXY010037781), was performed to find the sequences of kitlg and kit. EMBOSS est2genome program was used to identify exons and introns. The deduced mRNA sequences were cloned by PCR using ovarian and testis cDNA and specific primers designed to exons of the respective genomic sequences. The resulting amplicons were sequenced to confirm their identity.

Results

The Kit system consists of the growth factor kit ligand (or Stem Cell Factor) and its receptor c-kit. In mammals, among other functions, this system acts as regulator in several reproductive functions, especially during the early stages of gonadal development. The objective of the present work is to clone kit ligand (kitlg) and c-kit (kit) as a first step to study their involvement in the control of the initial stages of gonadal development in both males and females of European seabass, Dicentrarchus labrax, in order to increase the knowledge on the control of early stages of gametogenesis in fish.

Amino acid sequences of Kit system of European seabass and different vertebrate species (fish, frog, chicken and Human). Sequences were aligned using Clustal Omega and analyzed using the EMBL InterProScan program.

Phylogenetic trees based on amino acid alignment for Kit ligand and its receptors in fish, mammals, birds and amphibia using the Neighbor-Joining method with MEGA 7. Bootstrap test (1000 replicates).

Two forms of the kit receptor (kita and kitb) were found, but only one form of the kitlg was detected in European seabass.

Amino acid alignments and phylogenetic trees demonstrate that the cDNA sequences cloned from European seabass gonads encode for Kitlg, Kita and Kitb.

Tissue specificity analysis revealed expression of kitlg, kita and kitb in almost all tissues studied, including ovary and testis.

Expression of kitb is apparently higher than kita in the gonads of European seabass.

The distribution of expression patterns during gonadal development show evidences of apparently higher expression in males than in females and immature than mature gonads.

CONCLUSIONS

All these results strongly suggest that the Kit system plays an important role in gonadal development of the European seabass.

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