USE OF SOMATIC GENE TRANSFER FOR STUDYING GONADOTROPIN ACTIONS ON SPERMATOGENESIS IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX*).

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Introduction:

The follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) play central roles in vertebrate reproduction. These gonadotropins act by binding and activating specific receptors (FSHR and LHR) located in certain cell types of the fish gonads, and their action begins a signalling cascade controlling different steps of gametogenesis and steroidogenesis. Although the structural duality of these hormones is well defined, their individual roles are not clear in most fish species. Furthermore, promiscuous hormone-receptor interactions have been found in some species complicating our understanding of fish gonadotropin functions.

The availability and use of recombinant gonadotropins offers a unique tool for studying gonadotropin actions in a given species. One step further is the direct administration of the gonadotropin coding genes to the fish. This technique known as somatic gene transfer comprises a number of different approaches that aim to introduce a gene into an adult tissue. A broad range of delivery systems has been tested in mammals, where the application of this technique is mainly focused on gene therapy [1]. In fish, its development and improvement has been mainly directed to DNA vaccination. However, its application as a method for *in vivo* delivery of gene products into fish blood has hardly been explored [2].

We have developed a series of tools to study and control gonadotropin actions in the European sea bass. This species is a good model due to the already existing knowledge on its reproductive patterns and the availability of other tools for physiological studies. In addition, it is one of the most cultivated marine teleosts in the Mediterranean area.

Methods:

Animals: Mature adult male sea bass (3 years old) and prepubertal (1 year old) male sea bass. *Plasmids*: pCMVtk-Lacz and pcDNA3 (Invitrogen) were used as control plasmids. The gonadotropin expression plasmids, pcDNA3-scLH and pcDNA3-scFSH, contained sea bass single-chain (sc) gonadotropin cDNAs [3]. *Injections*: Intramuscular injection of the different plasmids was performed with an insulin syringe rostro-ventral to the dorsal fin. Uptake of the DNA by the muscle cells was potentiated by electroporation pulses. *Hormonal analysis*: Blood samples were taken through the study to evaluate FSH, LH [4] and 11-ketotestosterone [5] levels. *Histology*: Gonad sections were stained and testis developmental stages were classified according to [6]. BrdU incorporation was used to evaluate cell proliferation. *Sperm assessment*: Weekly samples of sperm were taken from the adult males to evaluate sperm volume and density. Gene *expression analysis*: Gonad samples from the injected animals taken at different intervals were used for RNA extraction. Variations in mRNA levels for different sea bass genes were measured by qPCR using Taqman chemistry and a specific standard curve for each gene.

Results and Discussion:

The availability of the genes coding for the sea bass gonadotropin subunits has enabled us to generate appropriate expression DNA constructs and recombinant gonadotropins in different eukaryotic expression systems [3], including the production of single-chain gonadotropins. All these recombinant hormones are functionally active, but their *in vivo* stability showed remarkable differences depending on the system used. Thus, somatic gene transfer has been evaluated as an alternative method for *in vivo* delivery of gonadotropins into fish blood. Intramuscular injections of the expression plasmids coding for a single-chain LH or FSH in sea bass produced a significant increase of the corresponding gonadotropins in the plasma. The effect of plasmid injection on gonadotropin plasma levels lasted longer than direct delivery of the recombinant hormone.

To test the functionality of the plasmid-derived gonadotropins, mature adult male sea bass were injected with the plasmid pcDNA3-scLH and we observed that the derived scLH was able to increase the production of sperm in the injected animals. On the other hand, immature yearling male sea bass were injected with pcDNA-scFSH to evaluate the functional effects of scFSH. The produced recombinant hormone was able to induce spermatogenesis in immature animals, as evaluated by histological and cell proliferation analysis. Besides, the expression of some germ cell markers, such as *scp3* or *piwi*-like, or potential target genes, such as the *LHR* was also assessed.

As a next step sequential injections of pcDNA-scFSH and/or pcDNA-scLH were performed in immature

yearling males to analyze the different and synergic actions of these hormones on spermatogenesis. After histological examination and qPCR expression analysis we can conclude that FSH but not LH is able to trigger spermatogenesis in sea bass, while LH potentiates the actions of FSH allowing a further progression of the spermatogenic process.

Conclusion:

The injection of the gonadotropin subunit genes into sea bass muscle produced functional hormones and has been used for basic studies on sea bass, revealing the differential actions of FSH and LH in spermatogenesis. Therefore, this approach represents a powerful tool for basic studies on gene function and a promising procedure for biotechnological applications.

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