ANIMAL GENOMICS

Molecular Cloning and In-Silico Analysis of A WGS derived genomic contig of a putative Angiotensinogen from the Teleost Sebastes Schlegelli.

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Angiotensinogen (AGT) is the major substrate in the Renin-Angiotensinogen system (RAS), the primary hormonal signaling cascade ascribed primarily towards body fluid and blood pressure regulation, with peripheral albeit salient pro-inflammatory immune roles.¹ A WGS derived genomic DNA contig sequence with a presumed angiotensinogen gene (3802bp with a 1383bp, 6-exon coding region) was acquired from Sebastes schlegelli (Rock Fish) and subjected to extensive computer-assisted sequence analysis. The polypeptide derived via sequence based prediction tools defined a length of 460 amino acids, with a molecular mass of 51.3KDA. Furthermore, RFAgt revealed a signal peptide incorporating approximately 19-residues upstream the putative angiotensinogen I signature motif (²⁰NRYYHPFYL²³), with the peptide cleavage site residing between ¹⁹Ala- Asp²⁰, indicating its secretory nature. RFAgt also demonstrated a Serpin domain (between residues 9-458) with conserved sequence motif (⁴³¹LSIPRPFFSV⁴⁴¹), implicating a sequence-specific non-inhibitory role.¹¹ Sequence homology and genetic distance based phylogenetic analysis (augmented by 1000-iteration bootstrap analysis) revealed that RFAgt is evolutionary proximate to the AGT’s of Oplegnathus fasciatus, Larimichthys crocea and Rhabdosargus sarba. Validation of the In-silico predicted ORF conducted via PCR amplification using sequence specific primers (F⁻⁵'-ATG CGG TCG CCT CTT CTA GC-³ and R⁻⁵'-TTA CAG TGT AGG ATT GAT GAT CTT GCC-³), and subsequent visualization via Gel-electrophoresis revealed a concomitant band at 1383 bp. Consecutively, upon purification, an attempt was made to ligate the product into a pGEM®-T Easy vector (size 3015bp). The experimental component will further expound on the Tissue-specific expression analysis with anticipated highest expression in the liver and a challenge (injury/infection) based expression study with a potential upregulation of RFAgt expression during physiological stress expected.¹¹

References