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Identification of Novel Halophilic Genes in Pufferfish

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Summary

Teleost fish maintain a similar salt content of their body fluids, approximately one-third of seawater (SW), despite of their external environments. Although strategies for maintaining body fluid homeostasis are different depending on their habitats, fundamental to osmoregulation is transport of water and ion in the gill and kidney. Furthermore, various hormones mediate osmotic signals from environment to these organs to regulate osmoregulatory capability. One of these hormones is neurohypophysial hormones, vasotocin (VT) and isotocin (IT) secreted from the hypothalamus. The aim of this study is to identify novel genes involved in ionic and osmotic regulation in pufferfish. Puffers include euryhaline and stenohaline species and are a useful model animal for studying the molecular mechanism of salinity tolerance by exploiting the fugu genome resources.

Four Tetraodontidae species, tiger puffer, grass puffer, green puffer, and *Tetraodon turgidus*, were transferred to four different osmotic conditions (SW, 33%SW, 10%SW, and fresh water (FW)). Their osmolality, $[Na^+]$ and $[Cl^-]$ of the blood and activities of Na^+, K^+-ATPase in the gill and kidney were assayed. These puffers showed different salinity tolerance depending on their habitats. In the tiger puffer transferred to FW, the renal Na^+, K^+-ATPase activity was increased concomitantly with the decrease of blood osmolality, $[Na^+]$ and $[Cl^-]$. Additionally, the amounts of VT and IT mRNAs in the hypothalamus were increased one day after transfer. Therefore, the kidney and hypothalamus of the FW-transferred tiger puffer were used in the screening of novel osmoregulatory genes.

The GeneFishing technology (Seegene, Inc.), which is an improved differential display method, was applied to identify novel genes. Three and two differentially expressed cDNAs were obtained from the kidney and hypothalamus, respectively. Two hypothalamic cDNAs (ACP9-H and ACP14-H) were cloned and their nucleotide sequences were determined. These two cDNAs encode 3' part of unknown mRNAs. Their expression profiles after transfer to FW were determined by real-time PCR. ACP9-H mRNA levels increased on day 1 and declined to half on day 2. ACP14-H mRNA levels decreased in response to salinity challenge, thus being a candidate of novel halophilic gene. Further identification of differentially expressed genes in tiger puffer and successive studies on their functions in puffers will provide valuable information on the molecular mechanism of salinity tolerance.