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VARIATION IN SELECTION CONSTRAINTS ON TELEOST TLRS WITH EMPHASIS ON THEIR REPERTOIRE IN THE WALKING CATFISH, CLARIAS BATRACHUS.

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ABSTRACT:

The high degree of conservation of toll-like receptors (TLRs), and yet their subtle variations for better adaptation of species in the host–pathogen arms race make them worthy candidates for understanding evolution. We have attempted to track the trend of TLR evolution in the most diverse vertebrate group—teleosts, where Clarias batrachus was given emphasis, considering its traits for terrestrial adaptation. Eleven C. batrachus TLRs (TLR1, 2, 3, 5, 7, 8 9, 13, 22, 25, 26) were identified in this study which clustered in proximity to its Siluriformes relative orthologues in the phylogenetic analysis of 228 TLRs from 25 teleosts. Ten TLRs (TLR1, 2, 3, 5, 7, 8 9, 13, 21, 22) with at least 15 member orthologues for each alignment were processed for selection pressure and coevolutionary analysis. TLR1, 7, 8 and 9 were found to be under positive selection in the alignment-wide test. TLR1 also showed maximum episodic diversification in its clades while the teleost group Eupercaria showed the maximum divergence in their TLR repertoire. Episodic diversification was evident in C. batrachus TLR1 and 7 alignments. These results present a strong evidence of a divergent TLR repertoire in teleosts which may be contributing towards species-specific variation in TLR functions.

INTRODUCTION:

At the molecular level, the immune system was steadily shaped by the local pathogen pressure that has led to a wide range of variation in immune responses, even within the organisms of the same vertebrate class. The toll-like receptors (TLRs) are such ancient sentinels of innate immunity that bind to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) to provide protection against pathogenic infections

and endogenous damage. They serve as excellent models for gaining an insight into host–pathogen interaction along the evolutionary timeline. TLR is comprised of three domains—an extracellular ligand recognition domain, a transmembrane domain and an intracellular toll/ interleukin-1 receptor (TIR) domain. The extracellular domain of each TLR is constituted of leucine-rich repeat (LRR) motifs which determine the ligand specificity of the TLR. However, despite high degree of structural conservation in the receptors, there are numerous reports on species-specific ligand recognition with respect to TLRs. This observation highlights the significance of extrinsic factors (ecological niche, feeding habits, microbial milieu of its environment, host genetics) that guide the selection constraints on the host receptor for its adaptation in the given environment. Phylogenetic analyses also reflect the evolutionary course of changes in immunity in response to surrounding microorganism.

RESULTS:

Courtship is preceded by highly aggressive encounters between males. Courtship and ating takes place in shallow waters between isolated pairs of males and females. The male lies in a U-shape curved around the head of the female, held for several second .An optimum result was found in induced spawning by using hormone at the dose of 0.8 ml kg-1 body weight to female followed by stripping method with higher rate of fertilization (80.4%) and hatching (84.1%). Further, frequency of some common morphological deformities (1.27-3.83%) was also recorded in the induced bred C. In Cell Cultures. Noga (1987) cultured the parasitic dinoflagellates aseptically using a combination of artificial seawater, mammalian cell culture medium, and GIB cells. The GIB cell line was initially isolated from gill tissues of the freshwater catfish, Clarias batrachus (see Noga and Hartman, 1981). The culture medium was later simplified; parasites grew and multiplied on GIB cells in a medium (I02/HBSS) with only mineral ions (Na, K, Ca, Cl, SO4, and PO4), glucose, and phenol red as a pH indicator (Noga, 1989). In the cultures held at 25°C the dinospores produced trophonts that fed on the cells in the monolayer (Figure 1.43). Three to four days later, they detached to form tomonts which sporulated and released new dinospores. These reinfected the cells in the cultures where they developed as trophonts. The cycle was repeated until all the GIB cells were killed. Each in vitro cycle required about a week for completion. The time for completion of a cycle of growth in culture was thus similar to the time for completion of a cycle on fish held at the same temperature (Lawler, 1980).

MATERIALS AND METHODS:

Data Retrieval:

The sequences of 228 TLRs from a total of 25 teleost species were analysed in this study (Supporting Data 1). The NCBI nucleotide database was used for the retrieval of TLR sequences from 24 species including Sparus Aurata, Ictalurus punctatus, Takifugu rubripes, Seriola lalandi/dumerii, Gadus morhua, Oreochromis niloticus, Carassius auratus/ carassius, Lateolabrax maculatus, Miichthys miiuy, Lates calcarifer, Trachinotus ovatus, Scophthalmus maximus, Epinephelus coioides, Salmo salar/trutta, Oncorhynchus mykiss, Danio rerio, Perca flavescens, Megalobroma amblycephala, Ctenopharyngodon Idella, Cyprinus carpio, Tachysurus fulvidraco, Oplegnathus fasciatus , Anabas testudineus and Pangasianodon hypophthalmus. The sequences and the accession numbers of TLR 1, 2, 3, 5, 7, 8, 9, 13, 21, 22 and fifteen fish-specific TLRs forming the miscellaneous category are listed in Supplementary Data 2–12. The sequences for C. batrachus TLRs were identified by conducting BLAST homology search against its reference genome (Assembly version—GCA_003987875.1) using the TLR sequences of its closest Siluriformes relatives, I. punctatus, T. fulvidraco and P. hypophthalamus as queries. Stringent cut-offs of E-value (< 1 × 10–5), percentage identity (> 80%) and query coverage (> 95%) were maintained to ensure the identification of C. batrachus TLR orthologues with potential full-length coding sequences. ORF finder from NCBI was used to detect the open reading frames and corresponding amino acid sequences from the identified C. batrachus scaffolds (Scaffold ids, ORFs and amino acid sequences listed in Supporting Data 13–24). Eleven C. batrachus TLRs with potential full-length coding sequences were identified by this approach including C. batrachus TLR1, 2, 3, 5, 7, 8, 9, 13, 22, 25 and 26. The sequence of C. batrachus TLR21 used in this analysis was extracted from NCBI Accession no. AGM39445.1.

DISCUSSION:

The present study aimed to study the adaptive evolution of teleost TLRs and consequently gain a deeper insight into the evolutionary trend of TLRs in Siluriformes species, C. batrachus. Phylogenetic analysis of the teleost TLRs depicted clustering of the 12 C. batrachus TLRs (TLR1, 2, 3, 5, 7, 8, 9, 13, 21, 22, 25, 26) with their respective orthologues in the five TLR subfamilies, thereby suggesting a high sequence-level conservation of teleost TLRs. The phylogenetic clustering of the Siluriformes and Cypriniformes TLRs in the inferred trees is in

congruence with the taxonomic proximity between the two groups. Despite that TLR4 has been identified in Siluriformes I. punctatus, P. hypophthalamus and T. fulvidraco, we did not detect a corresponding orthologue in C. batrachus via in silico approach14,19. Loss of TLR4 has been reported in several teleosts while its co-stimulatory molecules (CD14 and MD2) involved in activation of lipopolysaccharide (LPS) recognition pathway remain absent across all teleost genomes9. Alternate pathways for LPS recognition, mediated by other pathogen recognition receptors, have also been reported in some teleosts20. Though this evidence seems to justify the loss of TLR4 in some fish, yet, in order to get a complete picture of its evolution, further exploration is required to unveil the function of TLR4 orthologues in other teleost species.

Divergence, a well-cited teleost trait, is vividly reflected at the level of molecular evolution in TLRs21. Earlier, similar studies have suggested the occurrence of adaptive evolution in teleost V1R1 receptors, insulin genes and TUDOR domain containing protein 7 (Tdrd7)22,23. The evaluation of selection constraints on one of the most conserved vertebrate gene families, TLRs, indicates the role of both pervasive and episodic positive selection in shaping their current day repertoire. This is evident from the results of alignment-wide selection tests which detected episodic divergence in all the TLR alignments and a trend of positive selection for TLR1, 7, 8 and 9. Site-based selection also corroborated these results showing a higher number of positively selected codons for TLR1, 7, 8 and 9. While the divergent nature of TLR1 has been demonstrated in multiple vertebrate groups, the adaptive constraints on TLR7 family members vary widely24,25,26. Kloch et al. identified contrasting pressure of selection on rodent TLR1 versus TLR7 and 927. A report on avian TLR3 and 7 detected purifying selection acting on both these genes28. Nonetheless, Park et al. and Areal et al. identified signatures of positive selection in TLR7 family members in mammals5,29. The relaxed selection constraints on TLR9 in teleosts and its subclade, Perciformes, has previously been reported by Chen et al. and Zhu et al., respectively30,31 . Our findings showed a stringency in selection constraints on nucleic acid sensing TLR3, 13, 21 and 22. This is in contrast with the results from a previous study in teleosts, where TLR21 and 22 seem to have evolved under positive selection32. This variation in findings may be due to the lower number of species orthologues included in this study. The constitution of species and their phylogenetic proximity is a critical factor in computation of dN/dS ratios. It is noteworthy that the nucleic acid sensing TLRs (TLR7, 8, 9) with a pan-vertebrate presence depict a trend of positive selection while those (TLR13,

21, 22) that have suffered species-specific loss along the vertebrate evolutionary timeline are under purifying selection. Interestingly, despite showing no alignment-wide evidence of positive selection, TLR13 in teleosts has a considerably high number of positively selected sites; which indicates a higher degree of episodic selection in TLR13. The deduction of multiple divergent leaf nodes via aBSREL analysis also corroborates this hypothesis. Considering that TLR13 was split from the TLR11 family due to its scc architecture to form a subfamily that also includes its paralogs TLR21, 22 and 234,11; it may be suggested that TLR13 may be a hotspot for duplication in teleosts, wherein partitioning of functions from the parent gene may have led to neofunctionalization of the duplicated TLR along the evolutionary timeline33. The variation in selection constraints of the teleost TLRs may be endowed to their diverse habitats ranging from marine, fresh water, estuarine to terrestrial. The degree of exposure to microorganisms is also enhanced due to ingestion of the surrounding water along with feed.

CONCLUSION:

The rostral neurohypophysis (NH) extends dorsal to the pars distalis, while the caudal part vertically penetrates into the PI and ramifies in its component. The RPD mainly consists of erythrosinophils and PgH-positive cells which are comparable to the lactotropes and corticotropes mentioned in the literature. In the nonbreeding season, acidophils are the predominant cells of the PPD and are largely confined to its dorsal aspect. The two types of cyanophils present in the PPD could not be differentiated with the various techniques used in this study. However, those cyanophils which increase in number and are active during the spawning season may be the gonadotropes, while those which are cytologically inactive may be the thyrotropes. The pars intermedia consists of PAS+ and PhH+ cells.

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