Teleost NOD-like receptors and their downstream signaling pathways: A brief review

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1. Outline of pathogen recognition receptors

Pathogen recognition receptors (PRRs) are germ-line encoded receptor proteins aimed to recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) resulting in mobilization of downstream signaling responses leading to inflammation, microbial destruction and eventually activation of adaptive immune responses. PAMPs are conserved molecular structures expressed on similar pathogenic microorganisms, and include peptidoglycans, lipids, bacterial DNA, viral RNA, nucleic acids. DAMPs on the other hand are non-pathogenic endogenous host-derived molecules, such as heat shock proteins (HSPs), high mobility group box 1 (HMGB1), adenosine triphosphate (ATP) and histones released by damaged or necrotic cells [1]. In addition to directly binding and activating the PRRs, some DAMPs are known to have different targets that in turn affect the signaling by PRRs (Figure 1). For example, uric acid indirectly activates NLRP3 via reactive oxygen species (ROS) induction [2] whereas ATP activates NLRP3 via P2X7R signaling, HMGB1 binds to DNA and augments activation of TLR9 via crosstalk with receptor for advanced glycation end products (RAGE) [1]. Hyaluronan also does not recognize TLR4 alone but a unique complex of TLR4 with molecules such as MD-2 and CD44 [3]. In addition to PAMPs and DAMPs, molecular patterns involved in the activation of PRRs are MAMPs (microbial-associated molecular patterns). MAMPs include virulence factors in addition to essential components of both commensals and pathogens, e.g., lipopolysaccharide (LPS), peptidoglycan, or nucleic acids [4]. There are several families of PRRs that are either membrane-bound or located intracellularly. Transmembrane PRRs in plasma membrane include toll-like receptors (TLRs) [5] and C-type lectin receptors (CLRs) [6]. Intracellular PRRs include retinoic acid inducible gene 1 (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), cytosolic DNA sensors (CDs), and the newly identified cytosolic absent in melanoma (AIM)-like receptors [7–10]. The current review is focused on NOD-like receptors or NLRs that act in tandem with other transmembrane and intracellular receptors to orchestrate an efficient host immune response against microbial invasions and endogenous danger signals. Members of the NLR family possess a C-terminal LRR (leucine-rich repeats) domain and a central NACHT domain [11]. The current review is focused on NOD-like receptors or NLRs that act in tandem with other transmembrane and intracellular receptors to orchestrate an efficient host immune response against microbial invasions and endogenous danger signals. Members of the NLR family possess a C-terminal LRR (leucine-rich repeats) domain and a central NACHT domain (named after NAIP, CIITA, HET-E, and TP1). In addition, many NLRs in metazoans also have a N-terminal effector domain.

In the 1990s, a large family of conserved genes that encode proteins characterized by nucleotide binding domain and leucine rich repeats

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ABSTRACT

Nucleotide-binding oligomerization domain-like receptors (NOD-like receptors or NLRs) are key members of the immune system that act as intracellular sentinels. These pathogen recognition receptors are essentially characterized by a central nucleotide binding domain and a C-terminal leucine rich repeat domain responsible for recognition of pathogen. Over the past decade, our understanding of teleost's NLRs has enhanced significantly although the signaling pathways remain to be elucidated. In this brief review, we have tried to decipher the structural and functional aspects of NLRs in teleost. The review also engages in illustrating the various downstream signaling pathways/molecules reported so far in fishes that enable the NLRs to act as important players in immune responses and defense mechanisms against pathogens. Importantly, we try to explore the lacunae in structural and mechanistic details of NLRs in the teleost that would help in identifying key areas in which research is needed to complete our understanding of NLRs and their structural and functional evolution.
(NBD-LRRs) was discovered in the animal kingdom. In human, NLR genes were named variously such as CATERPILLER [11,12], NODs [13], NOD-LRR [14,15], NACHT-LRR, [16] and NOD-like receptor [17,18], until the introduction of the standard nomenclature “NLR” in 2008 after the approval of the Human Genome Organisation (HUGO) Gene Nomenclature Committee and the Mouse Genomic Nomenclature Committee [19]. In teleosts, NLRs were first reported in 2008 by Laing et al. in zebrafish [20]. The next few sections describe the structural and functional aspects of NLRs in teleosts and compare it with information already available in mammals.

2. Diversity of NLRs

The members of the NLR family are reported to vary in different species across vertebrates. In mammals, the number of NLRs range from 23 in humans to more than 30 in mice [21,22]. Invertebrate NLRs are much higher in number, extending from 135 in sponges [23] to 200 in sea urchins [24]. Similarly, in teleosts, different NLR proteins have been shown with nearly 400 NLRs being identified in the zebrafish genome [25].

Structure of mammalian NLRs has been determined employing X-ray diffraction, solution nuclear magnetic resonance and electron microscopy [26]. They have a tri-domain architecture comprising of an amino-terminal effector-binding domain, a central nucleotide-binding domain termed NOD domain and a carboxy-terminal leucine-rich repeat (LRR) domain. NOD domain also referred as NACHT domain has three associated helical domains namely, helical domain 1 (HD1), winged helix domain (WHD), and helical domain 2 (HD2). The central NACHT domain is unique to NLRs and is critical for their oligomerization, while the C-terminal LRR domain serves to recognize PAMPs and associated danger signals. Ligand binding to LRR causes oligomerization of NACHT and leads to conformational changes in receptor such that N-terminal effector domains become accessible to interact with downstream signaling molecules or molecular adaptors [10].

Mammalian NLRs have been broadly classified according to their effector domain, phylogenetic relations and physiological functions. According to the HUGO classification depending upon their effector binding domain (EBD) at N-terminal, NLRs are categorized into NLR (MHCIACTVATR domain (AD) or CARD-like domain prior to the AD domain in splice variants), NLRB (Baculoviral IAP repeats, BIR), NLR (CARD domain), NLRP (pyrin domain, PYD) and NLRX (yet to be identified domain). Based on phylogenetic relations, NLR family is divided into NODs (NOD1, NOD2, NOD3/NLRC3, NOD4/NLRC5, NOD5/ NLRX1, CIITA), NLRPs (NLRP1-NLRP14) and IPAF (ICE-protease-activating factor e.g., IPAF/NLRC4 and NAIP) [27]. According to their physiological functions, Coutermarch-Ott et al. [28] introduced another system of grouping. First group includes inflammasome-forming NLRs (NLRP1,3,4,6,7,10,12) associated with production of proinflammatory cytokines IL-1β and IL-18 as well as cell death known as pyroptosis [28, 29]. Other groups are reproductive NLRs (NLRP2,4,5,7-9,11,13,14) involved in reproduction and embryogenesis [30,31], and regulatory NLRs having either positive (NOD1, NOD2) or negative (NLRP12, NLRX1, NLRC3) role in regulating diverse immune signaling pathways.

In non-mammalian vertebrates, studies are mostly confined to teleosts and meagrely explored in birds, amphibians and reptiles wherein the reports lack functional studies/signaling pathways. The last decade has seen a steep increase of reports in fish NLRs but a clear picture with

Figure 1. Schematic representation of NOD-like receptors and their signaling pathways in mammals and teleost. MAMPs, PAMPs and DAMPs are recognized by various NLRs. This leads to the activation of downstream signaling cascades resulting in inflammatory responses. (PAMPs- pathogen-associated molecular patterns, DAMPs- damage-associated molecular patterns, MAMPs-microbial-associated molecular patterns, dsRNA-double stranded RNA, LRR- leucine rich repeats, CARD- casapse activation and recruitment domains, NACHT-NAIP (neuronal apoptosis inhibitory protein), CIITA (MHC class II transcription activator), FISNA- fish-specific NACHT associated, STING- stimulator of interferon genes, NFKB- nuclear factor kappa-light-chain-enhancer of activated B cells, PI3K-phosphatidylinositol-3-kinase, AKT- Protein kinase B, mTOR- mammalian target of rapamycin, TLR- Toll-like receptor, IFN- interferon, MDA5- melanoma differentiation-associated protein 5, RIG-1- retinoid acid -inducible gene I, JNK- janus kinase, STAT- signal transducers and activators of transcription, VEGF- Vascular endothelial growth factor, MHC- major histocompatibility complex).
respect to nomenclature and signaling is still lacking. Initially it was believed that NLRs first appeared in teleosts via domain shuffling as presence of their orthologs could not be demonstrated following in silico analysis in classical invertebrate model organisms such as *Drosophila melanogaster*, *Ciona intestinalis* and *Caenorhabditis elegans* [11]. However, the discovery of NLRs in many classes of invertebrates indicated the evolutionary conservation of these cytosolic PRRs.

In cyclostomes, the most primitive vertebrate group, presence of 34 NLR genes has been reported in the genome of the sea lamprey [32]. In addition, recently, 9 NLR genes identified in the Far Eastern brook lamprey genome have been grouped into two subfamilies (NLRA and NLRC) based on comparative genomics and phylogenetic analysis [33]. The same study has also shown high expression of these genes in immune tissues and their significant response to bacterial/viral ligands. The presence of NLRs has been predicted in chondrichthyes such as grey bamboo shark [34], whale shark [35] and major ray-finned fishes like bichir, American paddlefish, alligator gar, spotted gar and bowfin [36] following in silico analysis. In teleosts, whole genome sequencing along with advanced bioinformatics tools has facilitated the identification of several NLR transcripts over the years (Table 1) having similar domain architecture to mammals. However, NLR classification in this extremely phenotypically diverse group that comprises more than half of extant vertebrates is at times confusing. In 2008, Laing et al. [20] provided a basic framework for classification wherein NLRs in zebrasfish were categorized into three subfamilies NLRA, NLRC and NLRX and suggested that these were orthologous to their mammalian counterparts. Thereafter, Rajendran et al. [38] re-classified the NLRs in channel catfish employing subfamily nomenclature used in zebrasfish. They reported 22 NLRs, 6 belonging to subfamily A (renamed as per HUGO nomenclature), 2 belonging to subfamily B, 11 belonging to NLR-C subfamily, and 3 NLRs that do not belong to any of these subfamilies. It is to be noted that except NLR-B2 in zebrasfish (CARD-NACHT) and NLR-B1 in miiuy croaker (CARD-NACHT-LRR) all other NLR-B genes lack C-terminal domain that is unique to the bony fishes phylogenetically relates to mammalian NOD3 [20]. In the following year, Sha and group [37] used the HUGO nomenclature [19] to name NLRs in channel catfish as NOD1, NOD2, NLRC3, NLRCS and NLRX1 and suggested that these were homologues to their mammalian counterparts. Thereafter, Rajendran et al. [38] re-classified the NLRs in channel catfish employing subfamily nomenclature used in zebrasfish. They reported 22 NLRs, 6 belonging to subfamily A (renamed as per HUGO nomenclature), 2 belonging to subfamily B, 11 belonging to NLR-C subfamily, and 3 NLRs that do not belong to any of these subfamilies. It is to be noted that except NLR-B2 in zebrasfish (CARD-NACHT) and NLR-B1 in miiuy croaker (CARD-NACHT-LRR) all other NLR-B genes lack C-terminal domain as well as C-terminal domains [20,38,39]. In zebrasfish, 6 genes belonging to the NLR-B subfamily were identified which clade with mammalian NALPs following phylogenetic analysis based on similarity in the region of NACHT domain. Despite the suggestion that NLR-B subfamily have structural similarity to mammalian NALPs/NLRPs, NLR-B genes do not contain pyrin domain distinctive of NALP genes thus the idea of these genes being functional equivalents to NALPs subfamily is debatable [38]. In addition to NLR-A, -B and -C, another subfamily NLRX1 with unknown effector domain has been identified in fugu [40] although Li et al. [41] suggested NLRX1 to be a part of the NLR-A subfamily. After that, HUGO nomenclature has been used invariably to classify NLRs in teleosts. It is to be noted that NOD1, NOD2, NLRC3, and NLRCS belonging to subfamily C in mammals have been grouped under subfamily A in teleosts. The recent discovery of mammalian homologues of NLRP1 and NLRP3 in zebrasfish [42,43] suggests the presence of a conserved inflammasome pathway. In the following subsections, we will focus on the NLRs that are actively involved in providing robust immune responses.

### 2.1. NLRs involved in inflammatory responses

Structural and functional aspects of NLRs implicated in mediating inflammatory responses are well characterized in mammals. NOD1 and NOD2 sharing one or two CARD domains are known to trigger immune defense in response to bacterial peptidoglycan via detecting muropeptides released from bacterial peptidoglycan (PG) [44,13,45-47]. The

| Table 1 |
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| Fish species | Members/ reported NLRs | Mammalian orthologs/ novel molecules | References |
| Zebrasfish | NLR1A | NOD1 | [20] |
| Zebrasfish | NLR2A | NOD2 | |
| Zebrasfish | NLR3A | NOD3 | |
| Zebrasfish | NLR4A | NOD4 | |
| Zebrasfish | NLR5A | NOD5 | |
| Zebrasfish | NLRB1- NLRB6 | NALPs/NLRPs | [20] |
| Zebrasfish | NLR1C- NLRG2 | Novel to teleosts | [20] |
| Zebrasfish | NLRP1 | NLRP1 | [42] |
| Zebrasfish | NLRP3 | NLRP3 | [43] |
| Common carp | NLRCl- NLRCl1 | Novel to teleosts | [38] |
| Gold fish | gfnod1 | NOD1 | [74] |
| Gold fish | gfnod2 | NOD2 | |
| Gold fish | gfNlrx1 | NLRX1 | |
| Japanese flounder/Olive flounder | NOD1 | NOD1 | [68] |
| NLRCS | NLRCS | [93] |
| NLRC3 | NLRC3 | [39] |
| Rohu | NOD1 | NOD1 | [69, 70] |
| Grass carp | NOD1 | NOD1 | [71] |
| Grass carp | NOD2 | NOD2 | |
| Miiuy croaker | NLRCl- NLRCl48 | Novel to teleosts | [100] |
| Miiuy croaker | NLRB1 and NLRB2 | NALPs/NLRPs | [97] |
| Mirgal | NOD1 | NOD1 | [73] |
| Orange-spotted grouper | NOD1 | NOD1 | [77] |
| Nile tilapia | NOD1 | NOD1 | [76] |
| Nile tilapia | NOD2 | NOD2 | |
| Nile tilapia | NLRCS | NLRCS | |
| Catla | NOD1 | NOD1 | [188] |
| Atlantic salmon | NLRCS | NLRCS | [92] |
| Rainbow trout | NOD2a | NOD2 | [67] |
| Rainbow trout | NOD2b | NOD2 | [189] |
| Rainbow trout | NOD1 | NOD1 | [88] |
| Rainbow trout | NLRCS | NLRCS | |
| Rainbow trout | NLRX1 | NLRX1 | |
| Asian seabass | NLRCS | NLRCS | [87] |
| Blunt snout bream | NLRCS | NLRCS | [98] |
| Turbot | NLRCS | NLRCS | [85] |
| Japanese pufferfish | NLRX1 | NLRX1 | |
| Japanese pufferfish | NHERLCl- NHERLCl3 | Novel to teleosts | [36] |
| Spotted snakehead | NOD1 | NOD1 | [191] |
| Ya-fish | NOD2 | NOD2 | [80] |
mutation in NOD2 has been associated with serious inflammatory diseases such as Crohn’s disease and Blau syndrome in humans [48-50], emphasizing its importance in immune responses. In addition, NOD1 and NOD2 transcripts have been detected in rat’s Sertoli cells and germ cells implying their role in reproduction [51]. NLRC5 and NLRC3 having a single N-terminal CARD domain share high structural similarity. They are believed to act as negative regulators of inflammatory immune responses [52-56] and are also reported to regulate adaptive immunity. NLRC4 reported only in mammals so far is not seen involved in sensing pathogens. Rather, it acts downstream of NAIPs (NLR family, apoptosis inhibitory proteins) to form inflammasome [57] that promotes activation of caspase-1 [58,59].

Unlike these receptors in which CARD domain is present at N-terminal, NALPs (NACHT-LRR-PYD-containing proteins) in mammals are characterized by the presence of PYD effector domain [60]. These receptors are activated by a variety of damage-associated molecular patterns (DAMPs) and microbial products including double-stranded RNA from bacteria and viruses [61], toxins [62], muramyl dipeptide (MDP) [63] and ATP [58]. Also, host-derived danger signals, such as uric acid crystals released by necrotic cells activate NALP receptors [64]. It is interesting to note that some of the NALPs play a pivotal role in inflammasome formation. Another member of the NLR family that could be envisioned to be involved in inflammatory responses is NLRX1. N-terminal of this receptor is yet to be characterized although the crystallographic data of human NLRX1 C-terminal fragment revealed presence of N-terminal helical domain (LRRNt), central leucine-rich repeat modules (LRRM) and a C-terminal three-helix bundle (LRRCT) [65]. This receptor is unique because it is the only NLR that is localized in mitochondria. Loss of function of NLRX1 has been linked to a variety of autoimmune diseases, including inflammation in CNS and bowel, colitis and cancer in mammals [66].

In teleosts, NOD2 and NOD1 have been extensively studied and their ubiquitous expression has been seen [67-70]. Moreover, infection studies involving bacterial ligands [71,68-70,72-76] and virus [71,67, 77,68-70,75] have reported change in basal NOD1 and NOD2 expression suggesting their critical role in host defense against bacteria and viruses [74]. Although the existence of multiple splicing variants of NOD1 have been reported in human databases, only one form of NOD1 has been reported in zebrafish via Western blotting [78]. It is noteworthy to mention that NOD2 splice variants similar to human NOD2 have been reported in zebrafish [79], rainbow trout [67] and Ya-fish [80]. In rainbow trout, the shorter transcript NOD2a encodes the normal form whereas NOD2b has a longer 5’ untranslated region (UTR) with incomplete first CARD domain. Splice variants are suggested to have a role in a harmonized defense response and variants with deletion might be involved in competitive inhibition of downstream signaling under-lying the normal form [80]. Despite its importance in immune responses, NOD2 has been reported as a pseudogene in pangolins [81], and absent in birds [82], amphibians [83] and reptiles [84] suggesting its functional replacement by other PRRs. For example, in contrast to mammalian NOD2 which recognizes MDP, in chicken the function has been replaced by NLRP3 [63]. It has been proposed that different environmental conditions resulting in unique selection pressures induce differentiation in structure and function among NLR family members [82].

Mammalian ortholog of NLRC3 termed as NOD3 in channel catfish [38] and NLRC3 in turbot [85], Nile Tilapia [76], Asian seabass [86], miiyu croaker [87] and rainbow trout [88] belonging to the NLR-A subfamily, is shown to contain one N-terminal CARD domain. In these species, the constitutive and ubiquitous expression of NLRC3 in immune tissues suggests its role in innate immunity. NLRC3 expression is reported to be upregulated in response to poly I/C [86-88] and bacterial ligands such as LPS and PGN [86,88,89] as well as during bacterial and viral infection in teleosts [89,90,87,39,85,76]. NLRC3 has been suggested to positively [88] as well as negatively influence inflammatory responses [86,89]. Interestingly, unlike mammals where no splice variants for NLRC3 are reported [91], two splice variants NL3Ca and NLRC3b in turbot [85], and two splice variants NO3Da and NO3Db [37,38] have been described.

Mammalian NLRC5 ortholog in teleosts contains either one CARD domain as seen in catfish [37] or no CARD domain as in Atlantic salmon.
zebrafish are reported having conserved and zebrafish [94]. Similar to humans, five transcript variants in miiuy croaker [87], Japanese flounder [93], rainbow trout [88], salmon [92] and Japanese flounder [93]. Its expression was shown to be B. Chuphal et al. (Receptor-interacting protein 2) adaptor protein, also known as RICK (RIP-like interacting clark kinase) [15]. Active RIP2 kinase leads to ubiquitination of NEMO (NF-κB essential modulator) and subsequent activation of NF-κB pathway. NF-κB induces the expression of several proinflammatory cytokines and growth factors [109–111] (Figure 3). In addition to inducing classical NF-κB pathway via RIP kinase, NOD2 is reported to modulate NF-κB signaling by interacting with TAK1 (transforming growth factor b-activated kinase 1) and GRIM-19 (gene associated with retinoid-IFN-induced mortality 19) [112,113]. The involvement of both, NOD1 and NOD2, have been shown in synthesis of anti-microbial peptides in response to bacterial stimuli [106,108,114]. Further, processing of pro-inflammatory cytokines through interaction with caspase-1 [115] and regulation of apoptotic pathway via CORT signals [116] and RIP2K mediated JNK pathway [117] have been attributed to NOD1 (Figure 3). Also, RIP2 has been shown to interact with ASC to activate caspase-1 under inflammatory conditions [118–120] and hence, NOD1/2 may be considered as a modulator of caspase-1 mediated apoptotic pathway.

As in case of mammals, the versatile immune regulator RIP2 is considered as a downstream adaptor molecule for NOD1/2 signaling in teleosts [77,69,70,73]. In zebrafish [121], mirgal [73], miiuy croaker [122,123] and goldfish [124], nod proteins after recognizing appropriate ligands are shown to participate in activation of NF-κB pathway via interaction with RIP2K. Additionally, NOD1 [125] and NOD2 [75] induces other PRRs involved in anti-viral responses such as RIG-1 and MDA5 (Figure 3). This interaction between NOD2, RIG-I and MDA5 allows NOD2 to take part in RLR-mediated signaling pathways and hence induce IFN promoter activity [75]. Moreover, the induction of IFN-y (IFN-gamma related gene) expression after bacterial and viral infections as reported in teleosts such as zebrafish [126], channel catfish [127], grass carp [71] and rohu [128] is contemplated to be regulated via NOD2 [129,71] (Figure 3). It is interesting to note that unlike mammals where NOD1 and NOD2 signaling is primarily implicated in mounting inflammation in response to bacterial ligands, NOD1 and NOD2 in teleost is reported to be involved during viral infection via induction of other PRRs. As far as involvement of the nodosome in processing of proinflammatory cytokines is considered, there is no evidence to suggest the activation of caspase 1 by nodosome. Nonetheless, the involvement of caspase 1 in processing of IL-1β is shown in zebrafish [130] and seabass [131] though it is still unclear whether the mature IL-1β after processing has a biologically active function [132].

3.2. NLRC5, NLRC4, NLRC5

NLRC3, C4 and C5 are known to regulate immune responses by interacting with various signaling molecules/adaptors. Mammalian NLRC3 is implicated in viral sensing and considered as a negative regulator of innate immunity as it is shown to inhibit type I IFN response. The receptor also regulates various inflammatory signaling pathways viz. STING [56,133], NF-κB and ERK [134,135], PI3K/AKT/mTOR [136–138], TCR [139] pathway (Figure 4). Further, NLRC3 is reported to inhibit toll-like signaling pathway by interfering with K63-linked ubiquitination of TRAF6 (TNF receptor-associated factor 6) which is a TLR signaling adaptor [55]. NLRC3 also competes with ASC for caspase-1 binding to inhibit inflammation [140,141] (Figure 4).

NLRC4 acts as an adaptor protein for NAIP and forms inflammasome in response to intracellular bacterial flagellin [142–145] as well as other virulence factors such as P3-like proteins of Type III bacterial secretion system [146]. Thereafter, procaspase-1 is recruited to induce the process of pyroptosis [147,148] (Figure 5). Also, CARD-CARD interaction of NLRC4 with procaspase-1 leads to processing of pro-inflammatory cytokines resulting in neutrophil recruitment [149,150]. Interestingly, in case of Casp1−/− mice, Naip5/NLRC4/ASC inflammasome activates caspase-8 and induces cell death in response to flagellin-positive bacteria, indicating the existence of an additional pathway which is NLRC4 dependent but caspase-1 independent [151]. NLRC5 induces...
transcription of MHC class I genes by regulation of chromatin remodeling in lymphocytes and several phagocytic cells. In addition, it inhibits activation of RIG-I, MDA5 and type I IFN production thus, affects viral infection [152,153] (Figure 4). The role of NLRC5 in antiviral signaling is further validated in primary human fibroblasts and several cell lines in which NLRC5 gene knockout showed significant decreased levels of IFN-α/β in response to virus [154,155]. Besides, NLRC5 has been shown to regulate NF-κB and IFN-I signaling pathways both positively [156] as well as negatively [53,157]. It also modulates other signaling pathways such as JAK2/STAT3 [158], AKT/VEGF-A [159], PI3K/AKT [160], and inflammasome activity [161] (Figure 4). Surprisingly, Kumar et al. [162] did not observe any role of NLRC5 in cytokine production in virus-infected macrophages and dendritic cells. The influence of NLRC5 in inflammatory pathways has also been seen in birds [163,164].

In teleosts, role of NLRC3 or NLRC3-like genes in viral infection has not been explored though systemic inflammation with higher expression of proinflammatory cytokines has been observed in NLRC3-like homozygous mutant zebrafish [94]. In another study, overexpression of zebrafish NLRC3-like 1 upon bacterial infection has shown a decrease in expression of proinflammatory cytokines [102]. These studies indicated a suppressive role of NLRC3 in inflammation in zebrafish. In contrast, NLRC3 or NLRC3-like protein in Japanese flounder positively regulates cytokine expression [90]. The presence of CARD domain in NLRC3 raises the possibility of its interaction with other downstream signaling components such as RIPK2 via CARD domain in a manner similar to NOD1 and NOOD2 (Figure 4). This assumption is supported by a study in zebrafish in which interaction is reported between NLRC3 and apoptosis-associated speck-like protein (ASC) containing a CARD domain.
(caspase activation and recruitment domain) (Figure 4). Similarly, zebrafish NLRC3-like 1 has been shown to interact with RIPK2 which in turn inhibit NOD1-mediated downstream signaling pathways and proinflammatory cytokine production [102]. With regard to NLRC4, in spite of the fact that it is involved in host defense, particularly against enteric pathogens in human with possible association with human auto inflammatory diseases such as infantile enterocolitis and macrophage activation syndrome [165], the presence of its orthologs has not been reported in fishes, herptiles and birds so far.

As far as role of NLRC5 in immune defense is concerned, overexpression of NLRC5 [93] or its isoform, zfNLRC5d [95] in zebrafish resulted in significant inhibition of SVCV (spring viraemia of carp virus) infection though it did not transactivate IFN1 and IFN3 in response to Poly (I:C) and LPS. This suggests that unlike mammals, NLRC5 in fishes is involved in an IFN-independent antiviral response possibly through transcriptional regulation of TLRs and NF-κB signaling (Figure 4). On the other hand, overexpression of zfNLRC5 isoforms contributed to negative regulation of antibacterial immune response, with the decreased expression of IκBα whose function has evolved from being NF-κB inhibitors to transcriptional coactivators, positively regulating gene transcription [95]. Thus, on the basis of preliminary evidence, we speculate that unlike mammals, NLRC3 and NLRC5 acts as positive regulators of immune responses in teleosts.

3.3. NLRX1

The mitochondrially located protein NLRX1 has atypical features and plays a crucial role in dampening inflammatory responses. NLRX1 has
been shown as a negative regulator of TRAF6-NF-κB signaling [166, 167], and RLH- as well as MAVS-mediated antiviral signaling [168]. Recent evidence indicates that NLRX1 causes degradation of MAVS leading to the negative regulation of IFN signaling pathway and promoting HCV infection [169]. However, NLRX1 is also shown to potentiate TNF-α/bacterial/double stranded RNA-induced ROS production in HeLa cells [170]. The direct interaction between C-terminal fragment of NLRX1 and RNA ligands (polyI:C and single stranded RNA) is reported crucial for NLRX1-mediated ROS activation. The synergistic role of NLRX1 in ROS production could be seen in light of the fact that NLRX1 interacts with a matrix-facing protein UQCRC2 of the respiratory chain complex III. Increased ROS generation has been reported to activate pro-inflammatory pathways such as NF-κB and JNK. Although the increased ROS production may seem contradictory to the anti-inflammatory role of NLRX1, it has been implicated that this actually leads to apoptosis which is a typical host response to viral infection and tumorigenesis [171]. Interestingly, unaltered antiviral and inflammatory gene expression in response to influenza A virus or intraperitoneal injection of poly (I:C) in NLRX1-deficient mice [172] raises question on pivotal role of this mitochondrial protein in immune responses.

In teleosts, NLRX1 functions as a suppressor of inflammatory response. In grass carp, similar to mammals, NLRX1 attenuates innate immunity by interacting with TRAF6. In the same study, enhanced gene expression of IRF3, IRF7, and IFN-1 following poly (I:C) stimulation has been observed in NLRX1 deficient grass carp kidney cell line [173]. In black carp, direct association of the NACHT domain of NLRX1 with MAVS has been seen as critical for negative regulation of antiviral signaling [174]. In a recent study in this fish, like mammals, a complex formation between NLRX1 and Tu translation elongation factor (TUFM) has been speculated to negatively regulate MAVS-mediated RLR/IFN signaling [175] (Figure 5).

3.4. NLRP

Depending on the signaling pathway, NLRPs are broadly divided into two categories. In canonical NLRPs such as NLRP1, formation of inflammasome is followed by the activation of caspase-1, whereas NLRP6 and NLRP12 follow a non-canonical pathway which is inflammasome-independent or may involve alternate caspases such as caspase-4, -5 and -11 [176]. NLRP3, the most widely studied member of this group, can follow either canonical or non-canonical signaling pathways depending upon the stimulus and type of tissue. In the canonical pathway, activation of NLRP by PAMPs/DAMPs results in recruitment and polymerisation of apoptosis-associated speck-like (ASC) adaptor molecules via homotypic interaction. In humans, the

![Figure 5. Signaling pathways of NLRX1. In response to PAMPs, NLRX1 interacts with UQCRC2 leading to ROS production and induction of NF-κB and JNK pathways. It also negatively regulates MAVS-mediated RLR signaling and TRAF6-NF-κB signaling. In teleosts, NLRX1 inhibit gene expression of IRFs and IFNs (denoted by red lines). (UQCRC2- Ubiquinol-Cytochrome C Reductase Core Protein 2, RLH-RIG-1-like helicases, MAVS- mitochondrial antiviral signaling protein, TRAF-6-Tumor necrosis factor receptor (TNFR)-associated factor 6, FN- interferon).](image-url)
CARD-CARD interaction between oligomerised prion-like ASC and pro-caspase-1 leads to formation of multi-protein complex known as inflammasome responsible for the processing and maturation of the proinflammatory cytokines, IL-1β and IL-18 [60,177] (Figure 6). ASC is critical for caspase-1 activation although human NLRP1 can directly recruit caspase-1 with/without interacting with ASC due to presence of C-terminal CARD domain [176]. Mouse NLRP1 has been well characterized and has been shown to be essential in regulating host immune response [178]. In case of non-canonical pathways involving caspase-1 independent activation of pyroptosis and/or secretion of IL-1β and IL-18 occurs that have wide effects on both, innate and adaptive immunity. Other than these two pathways, some of the NLRPs modulate MAPK and NF-κB signaling pathways in mice [176].

Unlike NLRP1 and NLRP3, less information is available about the other NLRP subfamily members such as NLRP6 and NLRP12. NLRP6 is a key molecule that links inflammation with gut microbiota by activating both canonical and non-canonical pathways, thereby influencing gastrointestinal inflammatory, infectious and neoplastic diseases in the case of mice and serves as a protective agent in liver in humans [179]. In addition, mice NLRP12 has been implicated in suppressing and acting as regulator of colonic microbiota [180]. Despite having a crucial role in immunity, the ligands sensed by NLRP6 and NLRP12 are yet to be identified [181,182]. Moreover, in addition to the reproductive role of human NLRP7 [183], contradictory roles in immunity have been reported, having both positive and negative effects on inflammasome responses [184].

In teleosts, Laing et al. [20] had shown that the NLR-B family clade with mammalian NALPs (NLRPs) though they do not take part in inflammation, probably due to missing domains. Later, Li et al. [42,43] has described NLRP1 and NLRP3 in zebrafish which are structurally homologous to mammalian NLRP1 and NLRP3. As far as signaling is concerned, NLRP1 and NLRP3 in zebrafish [42,43], and NLRP3 in Japanese flounder [185] are shown to initiate ASC dependent caspase activation leading to IL-1 beta processing. In addition, NLRP3rel along with adaptor protein ASC have been functionally characterized in goldfish and is suggested to have similar inflammasome signaling pathway as in mammals [186] (Figure 6). These studies in teleosts point towards the evolutionary conservation of inflammation signaling pathways.

4. Conclusion

NLRs have emerged as pivotal players that act in tandem with other PRRs to orchestrate an effective immune response. The evolutionary conservation of NLRs throughout the animal kingdom highlights their significance in host defense mechanisms. Nevertheless, depending upon the pathogenic challenges faced by organisms in their particular niche, these NLRs show structural and functional diversity. NLRs, in general,

![Figure 6. Signaling pathways of inflammasome forming NLRs. PAMPs interact with inflammasome forming NLRs (NAIP-NLRC4, NLRP1, NLRP3) as well as non-inflammasome forming NLRs such as NLRP6 which then activates pro-caspase-1 leading to inflammatory response. In non-canonical pathway, NAIP-NLRC4 interact with caspase-8 whereas NLRP6 and NLRP12 interact with caspase-4,5,11 to induce pyroptosis. In addition, in teleost, NLRP3rel has also been shown to follow similar inflammasome signaling pathways (denoted in red lines). (LTA- lipoteichoic acid, ATP- adenosine triphosphate, S. aureus- Staphylococcus aureus NAIP-neuronal apoptosis inhibitory protein, ASC- apoptosis-associated speck-like protein containing a CARD).](image-url)
are involved in translating pro-inflammatory responses but there are certain NLRs that act as negative regulators dampening the strong stimulatory responses, thus maintaining tissue homeostasis crucial for cell survival. Interestingly, NLRs in teleosts show a vast spectrum of variation in structural and functional aspect, and ligand recognition depending on species. Despite the close structural and functional similarities of some teatose NLRs with their mammalian counterparts, there are others such as NLR C-like receptor that do not have any mammalian homologs and are absent in all tetrapods. There is scarcity of literature on downstream signaling pathways and adaptor molecules of NLRs in teleost. To develop a thorough understanding of these receptors in regulation of immune responses, extensive studies in teleosts are required taking into account the signaling cascades and crosstalk with other PRRs and inflammatory pathways. The understanding of functional and structural aspects of NLRs in teleost is not only essential from an evolutionary point of view, but also because teleosts are an important economic resource. Extensive aquaculture predisposes fish to a variety of infectious diseases and deciphering signaling mechanisms underlying the activation of these receptors would provide promising opportunities for therapeutics.

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Conflict of interest
The authors declare no conflict of interest.

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