SCIENCE CHINA
Life Sciences

•RESEARCH HIGHLIGHT•

November 2022 Vol.65 No.11: 2335–2337
https://doi.org/10.1007/s11427-022-2112-0

The discovery of type IV interferon system revolutionizes interferon family and opens up a new frontier in jawed vertebrate immune defense

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Received March 16, 2022; accepted April 12, 2022; published online April 25, 2022

Citation: Su, J. (2022). The discovery of type IV interferon system revolutionizes interferon family and opens up a new frontier in jawed vertebrate immune defense. Sci China Life Sci 65, 2335–2337. https://doi.org/10.1007/s11427-022-2112-0

Recently, an original research discovers a new type of interferon (IFN) system in jawed vertebrates, including type IV IFN (IFN-υ or IFNU) and its receptors (IFN-υR1 and IL-10R2), by researchers from State Key Laboratory of Freshwater Ecology and Biotechnology Institute of Hydrobiology Chinese Academy of Sciences, Laboratory of Marine Biology and Biotechnology Pilot National Laboratory for Marine Science and Technology, Qingdao Agricultural University and Jimei University. Based on class II cytokine gene features, an unannotated gene (designated as IFN-υ (IFNU) after the following investigations) was identified in zebrafish genome (on Chr 24), sharing low identity (5.5%–17.1%) with known IFNs in zebrafish. IFNs can induce antiviral state through the interferon-stimulated genes (ISGs). Induction of ISGs and antiviral regulatory activity via IFN-υ *in vivo* were verified in overexpressed, knockout and knockdown zebrafish. For cytokines, receptors are essential for their functions. The 13 class II cytokine receptor knockdown experiments demonstrated that CRFB12 (IFN-υR1) and CRFB4 (IL-10R2) morpholino always block the expression of the inducible ISGs via IFN-υ. Furthermore, knockout experiments *in vivo* confirmed IFN-υR1 and IL-10R2 are the receptors of IFN-υ. The antiviral function and receptors of IFN-υ were also verified in clawed frog (*Xenopus laevis*) A6 cell line. According to BLAST and synteny analyses, IFN-υ widely exists in jawed vertebrates. Gnathostomata IFN-υ contain 4–5 coding exons, possess highly conserved locus from cartilaginous fishes to primitive mammals which is different from all other three-type IFNs, share a conserved sequence signature (CXXXXX[W/L]) at N-terminal region, show more protein structure resemblance with type I IFN than that with type II and type III IFNs at C-terminal region, and demonstrate low protein sequence identities with all other types of IFNs. Furthermore, the specific receptor IFN-υR1 was also widely identified in the corresponding jawed vertebrates with conserved locus, intron phase (1-2-1-0-1-0), structures, and functional sites for JAK binding and STAT activation. Based on the evolutionary phylogenetic tree, IFN-υ/IFN-υR1 and other IFN/IFN receptor genes might have diverged before the appearance of cartilaginous fish, have undergone independent evolution, and have formed specific ligand-receptor pairs. These findings were published in *Nature Communications* (Chen et al., 2022).

Infectious diseases seriously threaten health and life, especially viral infections, such as COVID-19, flu, and AIDS. IFNs play a pivotal role in immune defense against viral infections, which have been marketed for more than 30 years with powerful impact on the global therapeutic protein market due to their diversity in terms of biological activities. IFNs belong to class II α-helical cytokines along

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with IL-10 family, involved in innate and adaptive immunity, which orchestrate host immune responses via receptors on cytomembrane in jawed vertebrates. The first IFN was discovered in chickens in 1957. So far, no IFN homologous gene or structurally conservative IFN protein is discovered in invertebrates. RNA interfere system is considered as an important composition in the antiviral immunity in invertebrates. Nevertheless, functional IFN-like proteins have been reported in mollusc (CgIFNLP) and arthropods (Vago). Classically, IFNs are categorized into three types: type I (IFN-α, β, κ, ω, δ, ε, τ, ζ, μ, ν), II (IFN-γ), and III (IFN-λs) IFNs, based on their receptors, biological functions, genomic loci, and sequence/structural homology. Type I and type III IFNs are the primary antiviral cytokine lineages, while type II IFN mainly contributes toward defenses against bacterial, fungal and parasitic pathogens. All the three types of IFNs are discovered in cartilaginous fishes, amphibians, reptiles, birds and mammals (Secombes and Zou, 2017).

Fishes are the most diverse vertebrates, which possess more than half of the living vertebrate species (Gui et al., 2022). Teleosts contain over 26,000 described species, economically important to humans. Also they are crucial for evolutionary biology (Li et al., 2021). Up to date, type III IFN is not reported in teleosts. Type II IFN consists of two members: IFN-γ and IFN-γrel (unique member in teleosts) (Gan et al., 2020). Teleosts possess a highly diversified type I IFN repertoire, which is classified into three groups (I-III). Group I IFN is composed of 4 subgroups (IFNa, d, e, h), group II IFN comprises 2 subgroups (IFNb, c), and group III IFN only consists of one subgroup (IFNf). Every subgroup contains one to several members. Group I IFN exists in all the teleost species, whilst group II IFN limits to certain species/lineages, such as salmonids, cyprinids and some perciformes, and group III IFN is to date found only in salmonids within the teleosts and spotted gar (*Lepisosteus oculatus*, holostei) representing the unduplicated sister group of teleosts. All the three groups exist prior to the appearance to teleosts. Class II cytokine receptor family in teleosts are uniquely named as cytokine receptor family B (CRFB) with 17 members (Chen et al., 2022). Recently, an intriguing research finds that broad-spectrum potent direct bactericidal type I IFN members widely exist in nonmammalian Gnathostomata, which bridge and unify the direct bactericidal and indirect antiviral (regulatory) bifunction of type I IFN in nonmammalian jawed vertebrates (Xiao et al., 2021).

The discovery and clarification of type IV IFN system (IFN and receptors) in jawed vertebrates (from cartilaginous fish to primitive mammals) adds a new type of IFN and revolutionizes IFN family. Up to date, IFNs are classified into four types: type I, II, III and IV IFNs. The co-existence and independent phylogenetic relationship of four types of IFNs indicate the uniqueness in jawed vertebrates (Figure 1). Type IV IFN shows the similar C-terminal sequence with type I IFN, and shares the signal-transduction receptor IL-10R2 with type III IFN, which imply that type IV IFN and type I/III IFNs originate from a common ancestor. In mammals, IFN-υ and IFN-υR1 only exist in Monotremata (primitive mammals), such as platypus (*Ornithorhynchus anatinus*) and Australian echidna (*Tachyglossus aculeatus*), but not in Metatheria and Eutheria, such as pig (*Sus scrofa*) and human (*Homo sapiens*), which may be the major reason why type IV IFN is identified till now. The study was supported with 6 figures, 29 supplementary figures and 6 sup-

<table>
<tr><th>IFN</th><th>Chondrichthyes</th><th>Teleost</th><th>Amphibian</th><th>Reptile</th><th>Avian</th><th colspan="2">Mammals</th></tr>
<tr><th></th><th></th><th></th><th></th><th></th><th></th><th>Primitive</th><th>Higher</th></tr>
<tr><td rowspan="2">I*</td><td colspan="2">Intron-containing</td><td>Co-existence</td><td colspan="3">Intronless</td></tr>
<tr><td colspan="6">Potent antiviral and direct bactericidal activities; IFNAR1/IFNAR2 receptors†</td></tr>
<tr><td rowspan="2">II</td><td>IFN-γ</td><td>IFN-γ, IFN-γrel</td><td colspan="4">IFN-γ</td></tr>
<tr><td colspan="6">Mainly defenses against bacterial, fungal and parasitic pathogens; IFNGR1/IFNGR2 receptors‡</td></tr>
<tr><td rowspan="2">III</td><td>IFN-λ; Intron-containing; Existence in some species</td><td rowspan="2">Absent</td><td colspan="4">IFN-λs§; Intron-containing</td></tr>
<tr><td>Similar to tetrapod</td><td colspan="4">Antiviral immunity; IFN-λR1/IL-10R2 receptors¶</td></tr>
<tr><td rowspan="2">IV</td><td colspan="5">IFN-υ; 4-5 coding exons</td><td rowspan="2">Absent</td></tr>
<tr><td colspan="5">Antiviral immunity; IFN-υR1/IL-10R2 receptors‖</td></tr>
</table>

Figure 1 Basic characteristics of four types of IFNs and receptors in jawed vertebrates. *, In placental mammals, type I IFN includes several subtypes, such as IFN-α, β, κ, ω, δ, ε, τ, ζ, μ, ν. In teleosts, type I IFN contains three groups. Group I consists of 4 subgroups (IFNa, d, e, h), group II is composed of 2 subgroups (IFNb, c), and group III only comprises one subgroup (IFNf). Every subgroup possesses one to several members. †, The phylogenetic and functional equivalents of IFNAR1 and IFNAR2 are CRFB5 and CRFB1/CRFB2 in teleosts respectively. The receptors for group I IFN are CRFB1 and CRFB5, while the receptors for group II IFN are CRFB2 and CRFB5 in teleosts. ‡, The homologs of IFNGR1 (IFN-γR1) and IFNGR2 (IFN-γR2) are IFNGR1-1 (CRFB17)/IFNGR1-2 (CRFB13) and IFNGR2 (CRFB6) in teleosts respectively. The receptors for IFN-γ are CRFB13 and CRFB6, while the receptors for IFN-γrel are CRFB17 and CRFB6 in teleosts. §, IFN-λs are also known as IL-28/29, four members (IFN-λ1 (IL-29), IFN-λ2 (IL-28A), IFN-λ3 (IL-28B) and IFN-λ4) in human. ¶, IFN-λR1 are also designated as IL-28R1. ‖, IFN-υR1 and IL-10R2 correspond to CRFB12 and CRFB4 in teleosts respectively.

plementary tables (Chen et al., 2022).

The investigations of type IV IFN system and its antiviral function open up a new frontier in jawed vertebrate immune defense. The following issues should be addressed in the future. (1) As an antiviral IFN and sharing the receptor IL-10R2 with type III IFN, how are the regulatory relationships (synergistic, independent or antagonistic) and regulatory signaling networks between type IV IFN and type I/III IFNs? (2) Except for the confirmed forceful antiviral immune function, whether type IV IFN exerts regulatory function to other pathogens (bacteria, fungi, parasites, etc.) like type II IFN? (3) On account of the similar C-terminal sequence with type I IFN and cluster with type I IFN firstly in the phylogenetic tree, whether type IV IFN has direct bactericidal activity like some type I IFN? (4) As a cytokine, whether type IV IFN regulates inflammation, phagocytosis, etc. in immune cells (lymphocytes, macrophages, dendritic cells, thrombocytes, neutrophils, etc.)? (5) Whether type IV IFN can be exploited as therapeutic protein?

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

References

Chen, S.N., Gan, Z., Hou, J., Yang, Y.C., Huang, L., Huang, B., Wang, S., and Nie, P. (2022). Identification and establishment of type IV interferon and the characterization of interferon-υ including its class II cytokine receptors IFN-υR1 and IL-10R2. Nat Commun 13, 999.

Gan, Z., Chen, S.N., Huang, B., Zou, J., and Nie, P. (2020). Fish type I and type II interferons: composition, receptor usage, production and function. Rev Aquacult 12, 773–804.

Gui, J.F., Zhou, L., and Li, X.Y. (2022). Rethinking fish biology and biotechnologies in the challenge era for burgeoning genome resources and strengthening food security. Water Biol Security 1, 100002.

Li, Y., Xiao, T., and Zou, J. (2021). Fish TNF and TNF receptors. Sci China Life Sci 64, 196–220.

Secombes, C.J., and Zou, J. (2017). Evolution of interferons and interferon receptors. Front Immunol 8, 209.

Xiao, X., Zhu, W., Zhang, Y., Liao, Z., Wu, C., Yang, C., Zhang, Y., Xiao, S., and Su, J. (2021). Broad-spectrum robust direct bactericidal activity of fish IFNφ1 reveals an antimicrobial peptide-like function for type I IFNs in vertebrates. J Immunol 206, 1337–1347.