综述

TLR5的进化、功能及相关疾病研究

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摘要 自第一个TLR蛋白被发现以来, 越来越多的TLR家族成员逐渐被鉴定出来。TLR5作为TLR家族中的重要一员, 是识别鞭毛蛋白的主要胞外受体。所有脊椎动物的TLR5在结构与功能上十分保守, 具有典型的TLR结构域, 即数量不等的亮氨酸重复序列、跨膜结构域, 以及细胞内的Toll/IL-1受体结构域。TLR5仅在鱼类中有两种形式, 分别为跨膜型TLR5M和可溶型TLR5S, 两者通过形成二聚体复合物, 将级联信号放大, 快速启动免疫反应。通过识别鞭毛蛋白, TLR5能够二聚化并激活级联信号, 引起促炎因子、抗炎因子或抗菌化合物的释放。因此, TLR5在保护宿主免受鞭毛病原体侵害以及维持或重建胃肠道稳态方面发挥了重要作用。尽管对TLR5的功能研究相对较少, 但在疾病的诊断与治疗过程中, TLR5/MyD88/NF-κB作为一条重要的信号通路, 具有宝贵的研究价值。关于TLR5的研究可为探索宿主–微生物的相互作用提供新的见解, 并为疾病预防和诊断提供重要的依据。

关键词 Toll样受体5; 进化; 信号通路; 炎症

Researches on the Evolution, Function, and Related Diseases of TLR5

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Abstract Since the discovery of the first TLR (Toll-like receptor) protein, several TLR family members have been identified. TLR5, an important member of the TLR family, is the major extracellular receptor that recognizes flagellin. TLR5 in vertebrates has highly conserved structure and function, with a typical TLR domain that consists of a variable number of leucine-rich repeats, a transmembrane domain, and an intracellular Toll/IL-1 recep-

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tor domain. It is only in fish that TLR5 has two forms, namely TLR5M (the membrane-anchored form TLR5) and TLR5S (the soluble form TLR5). Both TLR5M and TLR5S function by forming a dimer complex, amplifying the signaling cascade, and rapidly initiating the immune response. Upon flagellin ligation, TLR5 dimerizes and activates signaling cascades, leading to the release of pro- or anti-inflammatory cytokines or antibacterial compounds. Hence, TLR5 plays an essential role in providing host defense against flagellated pathogens and maintaining or re-establishing homeostasis in the gastrointestinal tract. Although there have been relatively few studies on TLR5 functions, TLR5/MyD88/NF-κB is found to be an important signaling pathway in the diagnosis and treatment of diseases, and it has great research value. Researches on TLR5 can provide new insights for exploring host-microbe interactions and may provide an important basis for disease prevention and diagnosis.

Keywords Toll-like receptor 5; evolution; signaling pathway; inflammation

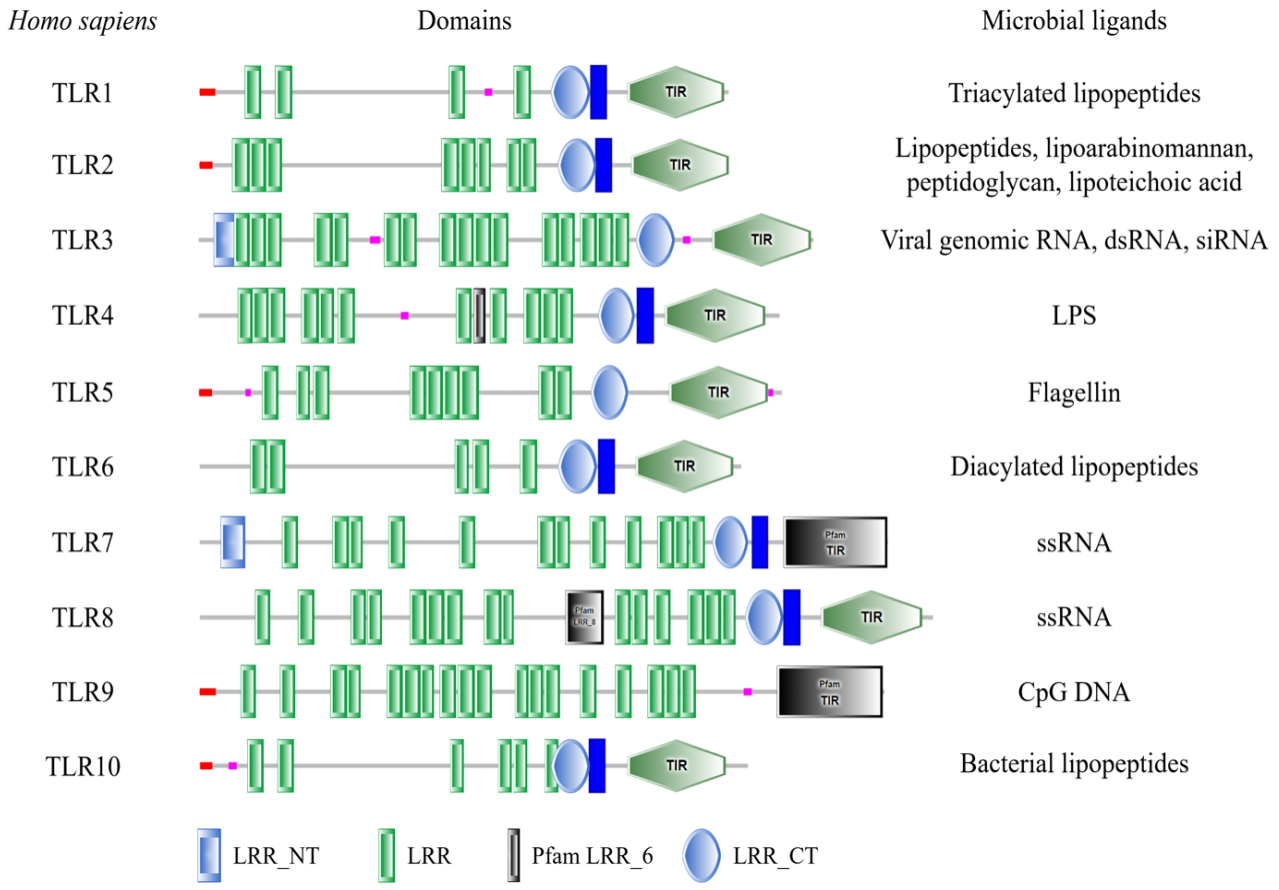
In 1985, ANDERSON et al[1] accidentally discovered a new type of *Drosophila melanogaster* gene and named it TLR (Toll-like receptor). Subsequently, LEMAITRE et al[2] found that changes in the TLR signaling pathway reduced the resistance of Drosophila to bacteria, resulting in a significant reduction in survival rate. It was believed that TLR had protective effects and might protect the body from pathogens[3]. Over the years, the TLR family received widespread attention because of its important roles in the immune system and several TLR family members were subsequently discovered[4]. TLR is a pattern recognition receptor and is highly conserved during evolution. Researchers have gradually discovered that TLR family members have slightly different expression patterns in different cells and tissues and recognize different ligands[5].

To date, 13 mammalian TLRs have been identified, of which 10 members (TLR1-10) are present in the human genome (Fig.1) and 13 in rodents (TLR1-13). Known TLR family members in various species have highly conserved structures that primarily include signal peptides located outside the cell and variable numbers of LRR (leucine-rich repeat) sequences, transmembrane domains, and intracellular TIR (Toll/IL-1 receptor) domains[6]. Crystallographic study of the extracellular regions revealed a solenoid horseshoes-like structure constituted by LRRs[2]. The LRR domain can specifically recognize conserved small-molecule motifs of PAMPs (pathogen-associated molecular patterns) and DAMPs (damage-associated molecular patterns) such as lipids, nucleic acids, proteins, and other products from microorganisms (e.g., bacteria, fungi, and viruses)[7-11]. The TIR functional domain is primarily responsible for signal transduction and can recruit downstream adapter molecules in the cell that also contain the TIR functional domain, thus achieving signal transduction. The adapter proteins primarily include MyD88 (myeloid differentiation primary response gene 88), MAL (MyD88-adaptor like protein), TRIF (TIR-domain-containing adaptor inducing interferon-β), TRAM (TRIF-related adaptor molecule), and SARM (sterile alpha and TIR motif-containing protein)[12-13].

Although numerous studies have investigated TLR evolution and function, limited systematic reviews of recent research advances on TLR5 are available. In the present review, we focus on the recent research progress on TLR5, the only TLR that senses flagellin, with respect to its evolution, function, TLR5 signal transduction pathway, and related diseases. We further investigate TLR5 as a potential therapeutic target and its application value in the diagnosis and treatment of diseases.

1 TLR5 and the signal regulation process

The human TLR5 consists of 858 amino acids and has a typical TLR structure. The extracellular domain is composed of 15 imperfect LRRs and a membrane-proximal LRR-CT (LRR C-terminal domain), constructing a solenoid horseshoes-like crystal structure (Fig.2A and Fig.2B). TLR5 is the major TLR



利用在线网站SMART(http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1)预测的人TLR结构域包括: TLR1(NP_003254.2)、TLR2(NP_001305716.1)、TLR3(NP_003256.1)、TLR4(NP_612564.1)、TLR5(BAB43955.1)、TLR6(NP_006059.2)、TLR7(NP_057646.1)、TLR8(NP_057694.2)、TLR9(NP_059138.1)、TLR10(NP_001017388.1)(括号内为GenBank登录号)。LRR: 富亮氨酸重复域; LRR-NT: LRR-氨基端结构域; LRR-CT: LRR-羧基端结构域; TIR: Toll/白介素1受体结构域; 红色矩形为信号肽; 蓝色矩形为跨膜结构域。

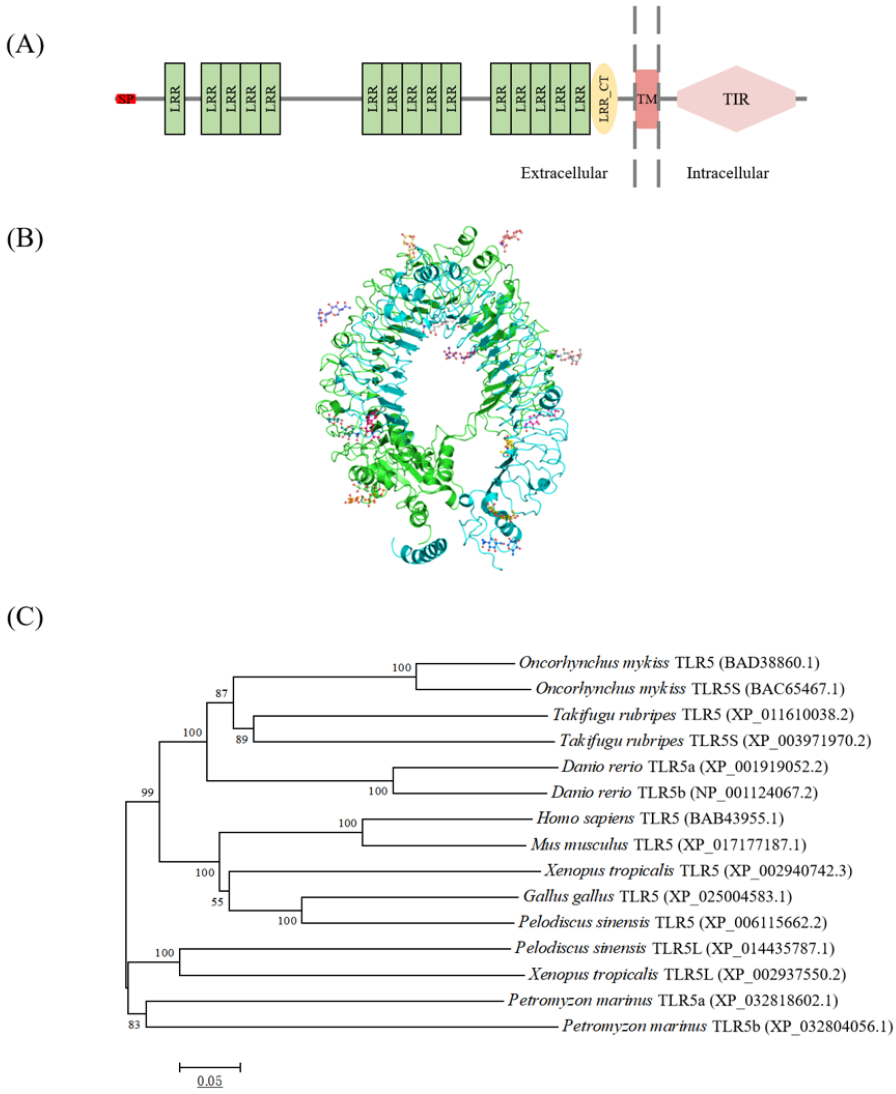
Schematic structures of the *Homo sapiens* TLRs were predicted by the online site SMART (http://smart.embl-heidelberg.de/smart/set_mode.cgi?NORMAL=1), including TLR1 (NP_003254.2), TLR2 (NP_001305716.1), TLR3 (NP_003256.1), TLR4 (NP_612564.1), TLR5 (BAB43955.1), TLR6 (NP_006059.2), TLR7 (NP_057646.1), TLR8 (NP_057694.2), TLR9 (NP_059138.1), and TLR10 (NP_001017388.1) (GenBank accession number in brackets). LRR: leucine-rich repeat; LRR-NT: LRR N-terminal domain; LRR-CT: LRR C-terminal domain; TIR: Toll/interleukin (IL)-1 receptor domain; the red rectangle: signal peptide; the blue rectangle: transmembrane domain.

图1 人TLR蛋白的预测结构域及各个TLR识别的微生物配体

Fig.1 Predicted domains and recognized microbial ligands of the *Homo sapiens* TLRs

member that can recognize bacterial flagellins. Conversely, bacterial flagellins such as those derived from *Salmonella* and *Pseudomonas aeruginosa* are also the only exogenous microbial ligands recognized by the currently known forms of TLR5[6]. Flagella are the organelles responsible for locomotion in bacteria[14]. Flagellin, a granular protein component of flagellar fibers, has highly conserved amino and carboxyl ends and may form the core structure of flagella[15]. In addition, it is a highly antigenic virulence factor recognized by the innate immune systems of several organisms[16].

When bacteria invade the body, TLR5 is responsible for recognizing bacterial flagellin via its LRR region and transducing the detected information as a signal to the downstream adapter protein MyD88 and initiate further signal transduction to downstream molecules. In mammals, TLR5 can also induce plasma B cells to differentiate into functional immunoglobulin A-producing cells through a specialized mechanism[17]. This is dependent on the activation of intracellular signal transduction pathways, which initiate the immune cascade and release inflammatory cytokines, thereby triggering the immune function of



A: TLR5的结构域示意图(根据参考文献[76]修改)。SP: 信号肽; LRR: 富亮氨酸重复域; LRR_CT: LRR_羧基端结构域; TM: 跨膜结构域; TIR: Toll/IL-1受体结构域。B: TLR5三级结构(PDB: 3j0a)。C: 采用MEGA7.0[77]邻接法构建的系统发育树(自展重复1 000次)。各节点处数字表示自展值。

A: the structure of TLR5 (modified from the reference [76]). SP: signal peptide; LRR: leucine-rich repeat domain; LRR_CT: LRR_C-terminal domain; TM: transmembrane domain; TIR: Toll/IL-1 receptor domain. B: the tertiary structure of TLR5 (PDB: 3j0a). C: the phylogenetic tree was constructed by the neighbor-joining method in MEGA7.0[77] (bootstrap=1 000). The bootstrapping values were showed as numbers at the nodes.

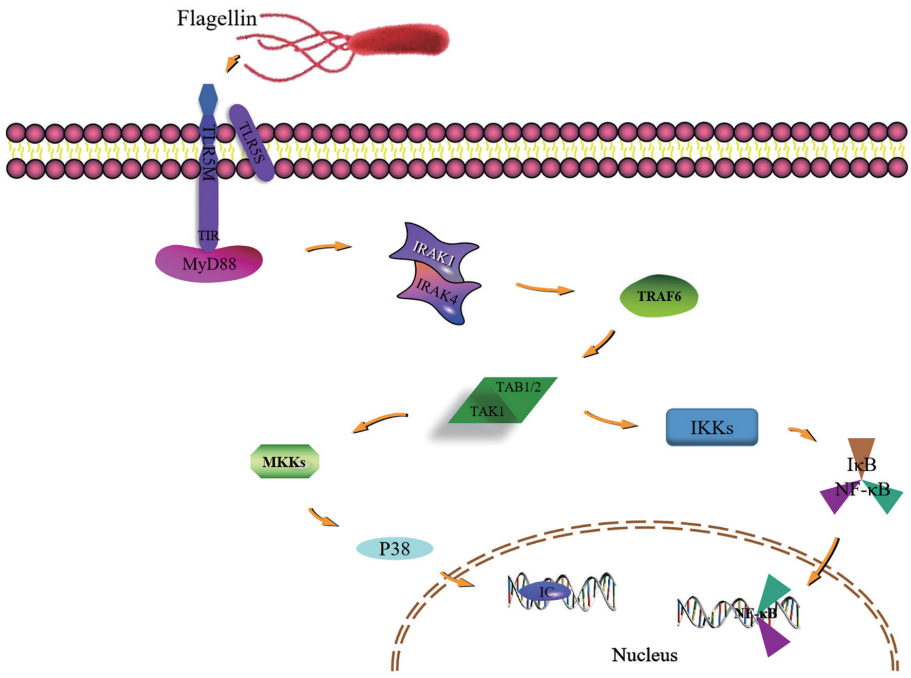
图2 TLR5的结构及系统发育树

Fig.2 Structure and phylogenetic tree of TLR5

TLR5[18-20]. The signal regulation process is shown in Fig.3[21-23].

2 Evolution of TLR5

Non-self-microbial ligands are recognized by TLRs in fish, amphibians, and terrestrial animals. Because of the multiple whole-genome duplications that occur in bony fish, they have many types of TLRs with diverse functions. It is speculated that because aquatic environments might have more complex bacterial species than terrestrial environments, fish needed more types of TLRs to resist invasion by exogenous pathogens, reduce the damage caused by various bacteria, and achieve healthy long-term survival[24]. Currently, at least 17 TLRs have been identified in fish and two types of TLR5 have been found: TLR5M (a membrane-anchored form TLR5) and TLR5S (a soluble form TLR5)[25-26]. TLR5S was first discovered in Japanese



MyD88: 髓样分化因子; IRAK: 白介素-1受体相关激酶; TRAF: 肿瘤坏死因子受体相关因子; TAB: 转化生长因子-β激活激酶-1结合蛋白; TAK: 转化生长因子-β激活激酶; MKK: 丝裂原激活蛋白激酶; P38: P38丝裂原激活蛋白激酶; IC: 炎症细胞因子; NF-κB: 核因子kappa B; IκB: NF-κB抑制因子; IKK: IκB激酶。

MyD88: myeloid differentiation primary response gene 88; IRAK: interleukin-1 receptor-associated kinase; TRAF: TNF receptor associated factor; TAB: transforming growth factor-β-activated kinase-1 binding protein; TAK: transforming growth factor-β-activated kinase; MKK: mitogen-activated protein kinase; P38: P38 mitogen-activated protein kinase; IC: inflammatory cytokine; NF-κB: nuclear factor kappa B; IκB: inhibitor of NF-κB; IKK: inhibitor-K-binding kinase.

图3 TLR5信号通路

Fig.3 TLR5 signaling pathway

puffer (*Takifugu rubripes*)[27] and was later identified in Atlantic salmon (*Salmo salar*)[28], channel catfish (*Ictalurus punctatus*)[29], and olive flounder (*Paralichthys olivaceus*)[30]. TLR5M has relatively intact transmembrane, LRR, and TIR domains, and is stably present in species with TLR5M; in contrast, TLR5S has only the LRR domains and lacks transmembrane and TIR domains. A study by TSUJITA et al[27] found that these two forms of TLR5 recognized flagellins and transduced signals downstream in unique ways. TLR5M is responsible for the recognition of flagellins. TLR5M also binds to TLR5S to pass the received message to TLR5S and amplifies it through a signaling cascade. The cascade reaction is completed through a MyD88-dependent pathway. In addition, it may induce the expression of other genes involved in the immune response, resulting in the activation of NF-κB and promoting the release of inflammatory factors[30]. To date, this specialized mode of flagellin recognition has only been reported in fish[31].

In fish with only TLR5M, signal transduction is achieved by the formation of a homodimer or heterodimer complex. Reportedly, TLR5 in zebrafish, grass carp, and other fish exists only as membrane-bound isoforms TLR5a and TLR5b[32]. It has been confirmed that duplication of TLR5 in zebrafish has concerted co-evolution, forming a unique heterodimeric complex[33]. Although the currently known TLR5 in *Schizothorax prenanti* exists as the membrane-anchored forms TLR5-1 and TLR5-2, changes in downstream gene expression could be detected to varying degrees after stimulation with flagellins. It is indicated that the formation of a heterodimer may not be necessary for function[9].

Amphibians represent a transitional stage between aquatic and terrestrial animals. They undergo

metamorphosis between adaptation to aquatic and terrestrial environments. In addition, the differences in the aquatic and terrestrial environments greatly challenge their immune system[34]. A total of 19 TLRs have been identified in amphibians, including fish-type and mammalian-type TLRs. It is speculated that amphibians need to resist various pathogens in the aquatic environment during the larval phase as well as other pathogens in the terrestrial environment after metamorphosis. However, there is only one kind of TLR5 in amphibians and similar to the immune defense mechanism of mammals and fish. Different TLR repertoire is the adaptation to their living environments in various vertebrates[35].

In the middle Mesozoic period, reptiles and mammals evolved independently. Reptiles are a large clade of vertebrates and the only poikilothermic amniotes, which occupy an important position in the evolutionary process[36]. The evolution of reptiles has improved the immune systems of their descendants. The discovery of reptilian TLR5 by VOOGDT et al[37] filled the gaps in the understanding of the evolution and function of vertebrate TLR5 and introduced a new avenue for studying the immune system of reptiles. However, the interaction between reptilian immune systems and microorganisms requires to be investigated in depth.

Studies have shown that TLR5 and its functional duplicates are not found in several mammals (such as guinea pigs, Yangtze river dolphin, pangolins, and pinnipeds)[38]. It is believed that a loss event occurred during the evolutionary process, which weakens the ability of the species to recognize flagellins[39]. However, because inactivation of TLR5 improves the survival of patients with melioidosis[40], it is speculated that the loss of TLR5 in guinea pigs, Yangtze river dolphin, pangolins, and pinnipeds is an evolutionary manifestation. A study on the replacement rate of TLR5 in different mammalian groups by PINHEIRO et al[41] showed differences in the evolutionary rate of TLR5 in different clades; in particular, the evolutionary rate of TLR5 in orders Lagomorpha, Rodentia, Carnivora, and Chiroptera is higher than that in other mammalian clades. Although the evolutionary process of TLR5 is complex, analysis of its structure has shown no significant overall changes in other mammals, underscoring that TLR5 is highly conserved throughout its evolutionary process[42]. The development of TLR5 is driven by the occurrence of gene duplication, loss, and conversion events[43-44].

To survive and avoid predators, birds have evolved rich and extensive ecological adaptations that have many similarities with those of mammals. The immune systems of both birds and mammals have similar characteristics, suggesting that certain functions are conserved[45-46]. Gene duplication may provide an opportunity for the evolutionary development of genes, enabling the production of either new genes or new functions in existing genes[47-48]. *TLR5* in more different avian taxa has developed into a pseudogene and exists in several species in a non-functional state. The lack of TLR5 in avian taxa and its effects on their immune systems require further investigation[49].

The structural similarities in fish TLR5M and mammalian TLR5 indicate that they are highly conserved in function from fish to mammals and may share a common ancestral gene origin[27] (Fig.2C). According to previous study[50], a homolog of TLR5 has been annotated from the genome assembly of lamprey. To date, no homolog of TLR5 has been reported in invertebrate, further suggesting that TLR5 first emerges in jawless vertebrates. The conservative evolution of TLR5 within the TLR family underscores its importance in the evolutionary process[37].

3 Function of TLR5

Investigating the underlying mechanism and signaling pathways by which the two forms of fish TLR5 recruit downstream transcription factors to promote health recovery has become essential. The concerted effects of TLR5M and TLR5S accelerate the signal amplification cascade and lead to rapid response against pathogens. In fish with only TLR5M, two proteins form a dimer and play important role as a pattern recognition receptor[33].

Upon binding bacterial flagellin, TLR5 stimulates the expression of proinflammatory, antibacterial and stress-related genes and hence plays an important role in the host defense against bacterial pathogens. *TLR5*-deficient mice do not show an immune response upon stimulation with flagellin[51]. In addition, *TLR5*$^{-/-}$ mice impaires the transport of pathogenic *S. typhimurium* from the intestinal tract to mesenteric lymph nodes[52] and are more susceptible to *Escherichia coli* infection in the urinary tract[53]. These results suggest that TLR5 is essential for the recognition of extracellular bacterial flagellin. Previously, researchers believed that TLR5 might play different roles in different vertebrates and that recognition of flagellins was species-specific. However, only the manifestation of TLR5 activity was found to differ in various animals[54]. In mice injected with flagellin, high TLR5 activity was observed, and it induced an increase in the release of the downstream IL-8; however, in chickens, the level of IL-8 release induced by TLR5 was significantly reduced[55].

TLR5 is abundantly expressed in virtually most types of epithelial cells from various mucosal organs especially on the basolateral surface of the intestinal epithelium[18,56] and can mediate various functions in the vertebrate intestinal tract. For example, it can help maintain the microbial environment and maintain metabolic balance in the body, which has a profound impact on intestinal homeostasis and health[57]. It is observed that *TLR5* knockout mice exhibited decreased *TLR5*-mediated gene expression and increased bacterial burden in the colon, which resulted in inability of mice to manage their commensal microflora and development of spontaneous colitis[58]. In addition, it helps promote metabolism and constantly accelerates the self-renewal of substances and energy in the organism. VIJAY-KUMAR et al[59] found that intestinal diseases of different degrees occur in mice lacking *TLR5*. Diseased mice had higher weight and significantly higher fat content than healthy mice, indicating induction of metabolic syndrome. After food restriction, the presentation of metabolic syndrome improved significantly, but that of hyperglycemia remained unaffected. This suggests that loss of *TLR5* reduces the release of inflammatory factors, resulting in changes in the community structure of intestinal microbes and preventing obesity caused by *TLR5* deficiency. *Proteus mirabilis*, which can cause chronic colitis, is detected in the intestine in the absence of *TLR5*, suggesting that it is closely associated with inflammation[60]. This shows that TLR5 can stabilize the microbial environment and play an important role in the potential control of bacterial populations. Although the structure of TLR5 is conserved through evolution, the available information about its associated functions is limited and more detailed and in-depth research is needed.

4 TLR5 and diseases

Inflammation is a form of defensive reaction that occurs when the body is invaded by exogenous inflammatory factors. It is a complex protective measure by which the immune system removes harmful stimuli from the body and repairs its tissues. During uncontrolled inflammatory reactions, TLRs act as initial pattern recognition receptors for PAMPs. The TLR/MyD88/NF-κB signaling pathway is an important pathway for regulating the immune system during the inflammatory response in the body, and it is widely distributed in the tissues and cells of various species. As the first barrier in the immune system, TLRs can sense and recognize various infections and pathogens. Additionally, TLRs participate in regulating the development of numerous inflammatory conditions, including infectious diseases, autoimmune diseases, and allergic responses. These activities of TLRs eventually lead to the development of an acquired immune response[61].

TLRs regulate the innate immune response to initiate and enhance host antibacterial and antiviral defense capacities. Flagellins are not only exogenous microbial ligands that stimulate the TLR5 pathway but also effective adjuvants involved in the innate immune response against many types of bacteria.

Therefore, flagellins are increasingly used as an exogenous activator to study TLR5 by triggering an inflammatory reaction in the body; this induces the release of a large number of cytokines in the lungs, intestine, liver, kidneys, and other tissues, thereby achieving the desired experimental effect[62]. UC (ulcerative colitis) is a type of intestinal disease whose incidence has gradually increased in recent years. It is a chronic disease that is affected by many factors and may be closely associated with the environment, immunity, and abnormalities in the intestinal flora[63]. By studying changes in TLRs, intestinal flora, and cytokines in patients with UC, MEENA et al[64] found that TLR2, TLR4, TLR5, and TLR9 expression levels were higher in patients with UC than those in the control group. In addition, blood IL-6 and TNF-α levels were also higher in patients than in the control group, indicating that TLRs and various inflammatory factors in the signaling pathway were closely associated with UC. Although the principal pathogenic mechanism of UC was not clearly defined in the study, changes in the intestinal flora and TLRs in patients indicated that the TLRs, which were involved in the disease, and associated cytokines in signal regulation pathways were clearly the influencing factors and provided a good theoretical target for disease prevention and treatment. In addition, through experiments on the regulation of MyD88-dependent pathways, LUBBAD et al[65] found that signal transduction pathways that affected TLR/NF-κB inhibited the release of downstream inflammatory factors, aiding the treatment of UC.

The invasion of an organism with flagellated bacteria has different effects on different tissues and organs. Therefore, a variety of inflammatory diseases including systemic lupus erythematosus, acute pneumonia, chronic enteritis, are closely associated with flagellin involvement[66]. The liver is an important organ in the process of sensing flagellin invasion. During the immunization process, high flagellin concentration can overactivate TLR5, resulting in the release of excessive inflammatory factors and thus cause liver injury. A study by YANG et al[67] showed that the involvement of flagellin resulted in the accumulation of many neutrophils and oxidative stress. Serum aminotransferase levels in the body were increased, leading to severe liver injury. In another study, XIAO et al[68] stimulated mice with different concentrations of flagellin and performed statistical analysis of the changes in different tissues. They found that transaminase levels in the liver were increased and TLR5 was excessively activated after the stimulation with high doses of flagellin, resulting in the release of many inflammatory factors and leading to severe liver injury with large areas of hepatocellular necrosis. In addition, high doses of flagellin could also induce hepatitis in mice in a short period of time. However, BURDELYA et al[69] found that low doses of flagellin could be used as a radioprotective agent during cancer radiotherapy and potentially improve the therapeutic index. This indicates that during immune activation of the TLR5 signaling pathway, flagellin involvement can be used as an experimental stimulus to study TLR5 and its signaling pathway. Therefore, further investigations to identify the breakthroughs of TLR5 in disease treatments are necessary.

5 Summary and prospects

In multicellular organisms, exogenous pathogens are common risk factors that can cause injury to tissues, organs, and other components of the body. The immune system provides an important safeguard and responds to environmental stimuli, broadens the niche, and ensures an advantageous evolutionary position for species[70]. The discovery of TLRs was a qualitative leap, raising awareness about the immune system[1]. As pattern recognition receptors, the TLR family clears exogenous microbial ligands and has played very important roles in the evolutionary history of the immune system.

During the evolutionary process, selective pressure exerted by microorganisms preserved the TLRs in various species and drove their diversification, thereby

creating a variety of TLR family members, recognizing different microbial ligands. As membrane-anchored proteins, TLRs are expressed on the cell surface and can also play protective roles in the cell. The TLR family of innate immune receptors is both evolutionarily conserved and capable of mutation[71-72]. The recognition of different PAMPs by the LRR domain determines the specificity of TLRs. TLRs sense the main components on the surface of different microorganisms, thereby exerting their antibacterial and anti-inflammatory effects[6,73]. The successive discovery of other TLRs has resulted in a very large TLR family, which has become a special taxonomic group in the immune system that can effectively identify invasive pathogens. To date, complete TLR families have been found and identified in many species; 27 TLR family members have been identified in vertebrates[74], including 10 TLRs (TLR1-10) in humans and 22 TLRs in fish[75]. Moreover, the discovery of TLR5 is interesting, as it not only improves our understanding of the TLR family but also expands the scope of recognizing microbials. TLR5M and TLR5S have improved our understanding of TLR5 and attracted increasing attention from researchers. Although TLR5 functions as an interactive dimer complex, over the course of evolution, only one membrane-anchored form of TLR5 has been found and identified in vertebrates other than fish. Besides playing an immune role in the body, it also maintains the balance of the microbial population in the intestine and promotes metabolic stability of the body[57-59]. Due to their specific living environment and evolutionary history, the TLR family in fish is more diverse than that in mammals, with a greater number and types of TLRs, which may improve the range and potential for recognition of PAMPs. Since TLRs have occupied an indispensable position in evolutionary history, their associated biological processes need to be further elucidated.

TLR5/MyD88/NF-κB is one of the key signaling pathways involved in the immune response during disease diagnosis and treatment. Flagellin can be used not only as a stimulus for the activation of TLR5 signaling pathway, but also as an adjunctive medication in cancer radiotherapy[69]. Therefore, studying this pathway can improve the potential application value of flagellin. Each member of the TLR family recognizes different microbial products and protects the body from microbes in many diverse ways. Based on this, associated factors in the signal transduction pathway can be identified as disease prevention and treatment targets and used to develop further treatment for the prevention of disease development and progression.

The discovery of TLR5 is undoubtedly of great significance, but it faces many challenges in its application to disease diagnosis and treatment. First, the available studies on its function are not very thorough, and its mechanism of action needs further investigation. Second, although appropriate flagellin intervention can be used as an adjunct to cancer treatment and potentially reduce pain in patients with cancer, there is no direct evidence for the direct involvement of flagellin in the prevention and treatment of cancer. Third, the imbalance of TLR5 leads to enteritis, liver function abnormality, and other diseases. Thus, there are research questions that need to be answered such as drug targeting of TLR5 signaling. Fourth, the possibility of toxic and other adverse effects during clinical application cannot be neglected. In conclusion, the progress in research on the evolution, function, and disease treatment strategies of TLR5 underscores its important research value, and further in-depth investigations of its functional mechanism will surely provide new perspectives on the evolution of the vertebrate immune system as well as disease treatments.

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