Nucleotide-binding oligomerization domain 2

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Nucleotide-binding oligomerization domain 2 (NOD2) is a cytoplasmic receptor that recognizes invading molecules and danger signals inside the cells.

NOD2 NLRs ER stress autophagy innate immunity 1. Introduction

1. Introduction

The innate immune system provides the first line of defense against danger and relies primarily on pathogen recognition receptors (PRRs) to do so [1]. PRRs are the immune system players that recognize the molecules frequently found in pathogens or released by damaged cells (respectively known as pathogen-/damage-associated molecular patterns (PAMPs or DAMPs)) [2].

PRRs are categorized into four distinct functional groups: (1) Toll-like receptors (TLRs), (2) retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), (3) C-type lectin receptors (CLRs), and (4) nucleotide-binding oligomerization domain-like receptors (NLR) [3].

NLRs are intracellular immune receptors conserved in both animals and plants. While some NLR proteins are involved in early embryogenesis and regulate the expression of major histocompatibility complex (MHC) molecules, certain NLR proteins play critical roles in recognizing damage-associated molecular patterns and in triggering immune responses [4].

The major PPRs, including TLRs, detect and capture pathogens on the cell surface or within endosomes, while NLRs are cytoplasmic receptors and detect their ligands in the cytosol, thereby providing another level of cell protection [5].

Nucleotide Binding Oligomerization Domain Containing 2 (NOD2) is a well-known member of the NLR family, which is expressed primarily in immune and epithelial cells [6][7]. This receptor detects a fragment of bacterial cell wall known as peptidoglycan muramyl dipeptide (MDP) and subsequently activates the signaling pathways, leading to proinflammatory cytokine production [8]. It has been shown that the polymorphisms in the NOD2 gene contribute to failure in microbial detection and are associated with increased susceptibility to some infectious diseases and granulomatous inflammation [9].

2. NLR Family and Structure

NOD-like receptors (NLRs) are evolutionally conserved proteins, belonging to the PRR family [5]. NLRs are also considered a large family of cytoplasmic receptors consisting of 22 members in humans and 34 members in mice [10]. They have an important role in the triggering and development of innate immune responses thorough sensing intracellular danger signals [11].

NLR proteins share a conserved triple domain structure containing a C-terminal leucine-rich repeat (LRR) domain, a central nucleotide-binding and oligomerization domain (NOD/NBD) (also known as NACHT domain), and a N-terminal protein–protein interaction domain (**Figure 1**) [7]. The C-terminal LRR domain is responsible for the detection of PAMPs and DAMPs and negatively regulates protein activity. The central NOD domain has ATPase and nucleotide binding activity, which is critical in protein oligomerization and function. The NOD domain contains a proximal helical domain 1 (HD1), a distal helical domain 2 (HD1), and a winged helical domain (WHD) [7] (**Figure 1**). The N-terminal effector domain is responsible for interacting with the downstream signaling molecules.

Based on the type of effector domains, the NLR family is divided into several subfamilies including NLRA containing an acidic transactivation domain (AD), NLRB (also known as NAIP) with a Baculovirus IAP Repeat (BIR) domain, NLRC with a caspase activation and recruitment domain (CARD), and NLRP with PYRIN domains (PYD) [12].

The NLRA and B subfamilies are involved in antiapoptotic functions and the transcription activation of MHCII via their intrinsic acetyl transferase (AT) activity. NLRC is one of the largest subfamilies of NLRs, consisting of six members (NOD1-5 and class II trans activators) that are characterized by their CARD effector domains [13].

The effector CARD domains have an important role in NLR's downstream functions and interact with other CARD-containing proteins through homophilic interactions. NOD2 contains two tandem CARD effector domains and can interact with a wide variety of proteins containing the CARD domain (**Figure 1**) [12]. Here, we provide a brief review of NOD2 mechanisms and functions.

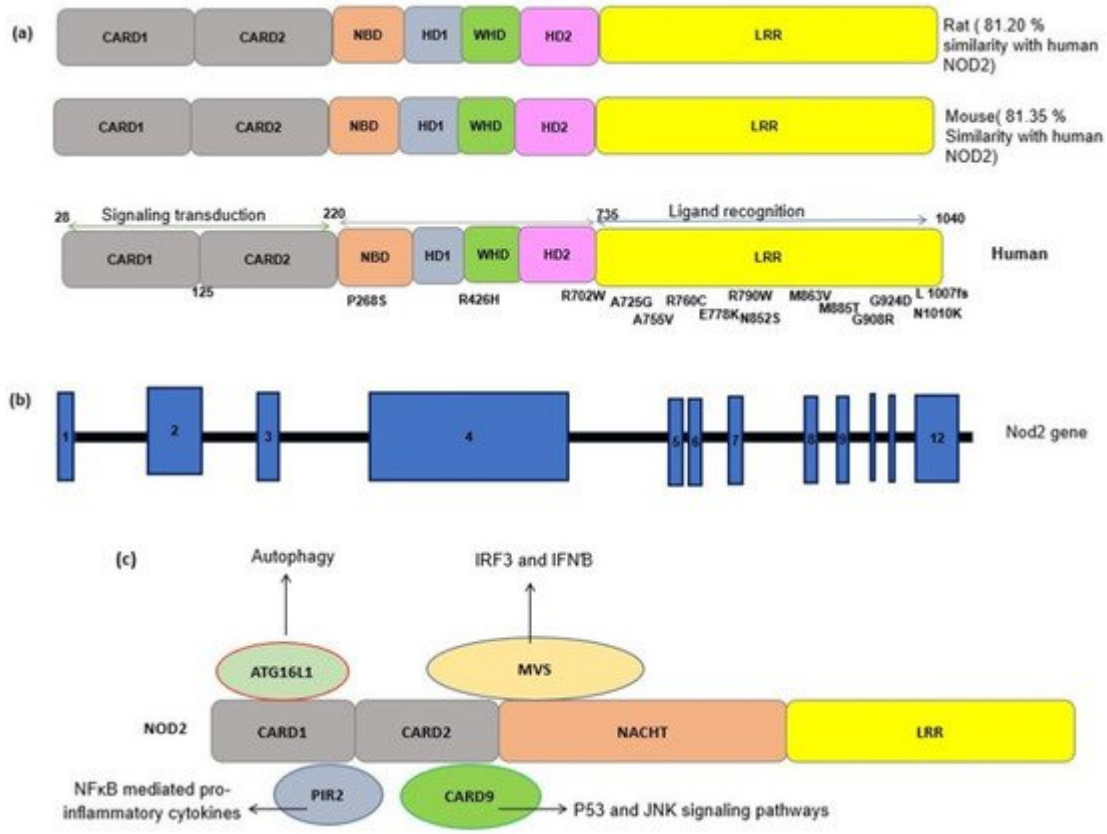

Figure 1. (**a**) Schematic representation of the NOD2 protein structure in a human, a mouse and a rat. The sequence identity of NOD2 gene in the rat and mouse with the human NOD2 gene is estimated by pairwise BLAST [14]. Common SNPs have been shown alongside the protein domains. CARD, caspase recruitment domain; NACHT, nucleoside triphosphates' (NTPase) domain; LRR, leucine-rich repeats. (**b**) A schematic representation of the NOD2 gene. NOD2 is composed of 12 exons (blue rectangles). The numbers inside the blue rectangles indicate the exon numbers. (**c**) A schematic of the interactions between NOD2 and other cellular proteins. The interaction of activated NOD2 with PIR2 activates the NF-kB pathway. The NOD2 interaction with Autophagy-related 16-like 1 (ATG16L1) induces autophagy machinery assembling. NOD2 interacts with the adapter protein mitochondrial antiviral signaling protein (MAVS) upon sensing ssRNA, active interferon-regulatory factor 3 (IRF3) and consequently production of interferon β (IFNβ).

3. NOD2: Cellular and Molecular Mechanisms

NOD2 acts through the mitogen-activated protein kinase (MAPK), inflammasome-associated, and NF-κB pathways which are considered the three main cell signal transduction pathways [15]. In the epithelial cells, NOD2 molecules are committed to the synthesis of anti-pathogenic peptides [16]. The expression level of specific antimicrobial α-defensins was significantly decreased in the Paneth cells of NOD2 knockdown mice [17]. Additionally, NOD2 can recruit Autophagy-related 16-like 1 (ATG16L1) and subsequently induce autophagy after activation (**Figure 1**) [18].

NOD2 is primarily activated upon sensing a component of bacterial peptidoglycan named N-acetyl muramyl dipeptide (MDP). It has been shown that NOD2 interacts with a wide variety of proteins. Mycobacterial N-glycolyl

muramyl dipeptide and viral ssRNA are also the ligands of NOD2, which can active their associated signaling pathways [2].

Until the ligand activation, NOD2 is maintained in an inactive, autoinhibited conformation in the cell through interactions of the NOD domain with LRR domains and cellular chaperones, such as heat shock protein 90 (HSP90).

Upon activation, the C-terminal LRR domain of NOD2 undergoes a conformational change and exposes the CARD domain, which allows it to interact and oligomerize with the CARD domain in the adaptor molecule RIP2 (receptor-interacting protein 2) through a homophilic interaction. Upon oligomerization, activated PIR2 applies lysine 63 (K63)-linked polyubiquitination at lysine 209 of the kinase domain. This ubiquitination promotes recruitment of TAK1 and NEMO (the NF-kB essential modulators). The activation of TAK1 and NEMO promotes phosphorylation of the IKKβ kinase, which is a key kinase in the NF-κB signaling pathway. Phosphorylated IKKβ degrades IκB and subsequently actives NF-κB family transcription factors and the production of inflammatory chemokines [19] (**Figure 2**).

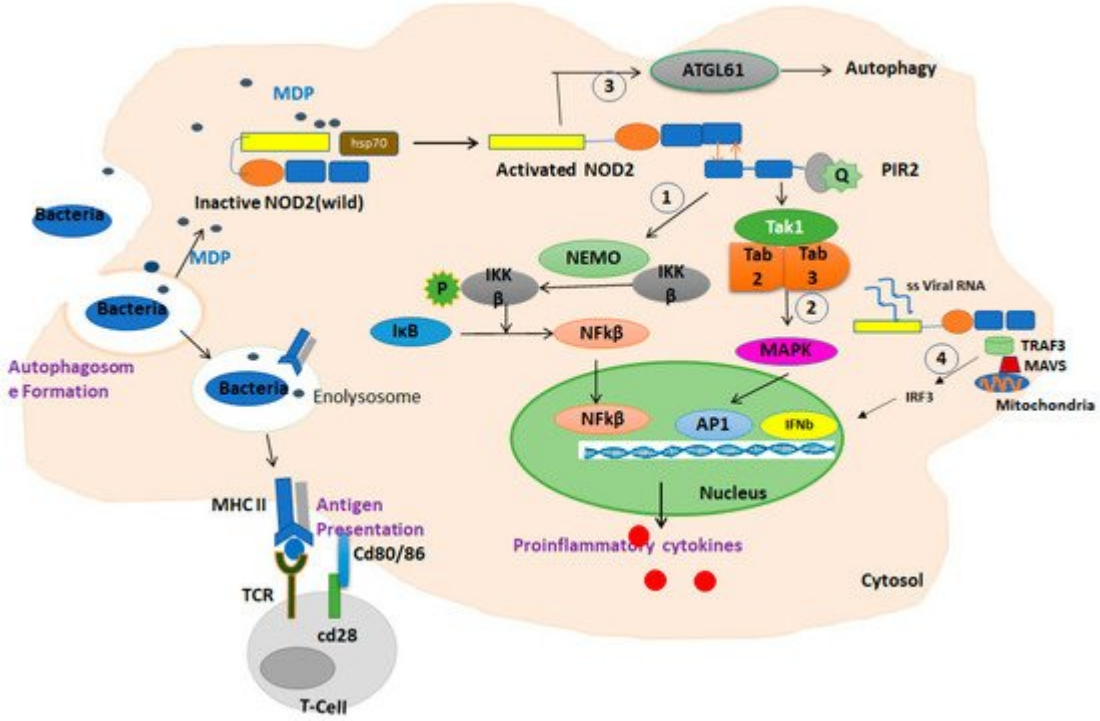

Figure 2. Summary of the function of NOD2 in response to MDP in macrophages. In the absence of a ligand, NOD2 is in an inactive autoinhibited form by folding the LRR domain onto NBD and CARD domains stabilized by chaperone proteins such as HSP70. Upon ligand binding and receptor activation, the C-terminal LRR domain of NOD2 undergoes a conformational change and exposes the CARD domain to interaction and oligomerization with the CARD domain in the adaptor molecule RIP2 through a homophilic CARD–CARD interaction. Upon oligomerization, PIR2 activates and promotes the ubiquitination of lysine 209 located at the kinase domain. (1) This ubiquitination promotes the recruitment of TAK1 and NEMO (the NF-kB essential modulators). The activation of TAK1 and NEMO promotes the phosphorylation of IKKβ, which is a key kinase in the NF-κB signaling pathway.

Phosphorylated IKKβ degrades IκB and subsequently activates NF-κB family transcription factors. (2) In addition, this path also activates the MAPKs and AP1 pathways. The NF-κB and MAPK pathways are responsible for triggering the expression of inflammatory cytokines. (3) Activated NOD2 also may trigger an autophagic pathway by recruiting ATG16L1. (4) The interaction of NOD2/TRAF3 with mitochondrial antiviral signaling protein (MAVS) upon sensing viral ssRNA induces the activation of IRF3, triggering the expression of IFN-β gene.

Conversely, the activation of NOD2 by viral ssRNA leads to the production of interferon β (IFNβ) through an alternative pathway by recruiting an adapter protein, a mitochondrial antiviral signaling protein (MAVS), and an activating interferon-regulatory factor 3 (IRF3) [20] (**Figure 1**).

NOD2 also may trigger an autophagic pathway upon the detection of bacterial MDP by recruiting ATG16L1 to the bacterial entry site, which results in the engulfment of invading bacteria by autophagosomes formation [21]. The NOD2 signaling pathways are summarized in **Figure 2**.

4. NOD2 Genetics and Polymorphism

The human gene encoding for the NOD2 receptor is CARD15, located on chromosome 16q12.1. The NOD2 protein has 104 amino acids with a molecular weight of 110 kDa, which is a multifunctional receptor. As NOD2 has many important roles, mutations in its gene may have serious consequences in vital cellular functions and immunity. NOD2 is a repository of genetic variants, most of which are associated with pathological conditions. Many previous studies have reported the association of NOD2 polymorphisms with inflammatory diseases (**Table 1**) [22]. As LRR is the ligand binding domain of the NOD2 receptor, mutations in this region may affect either responses to MDP or the downstream pathways [23]. The nonsense mutations in this region also may abolish the conformational changes needed for MDP binding and receptor activation and thus may lead to receptor loss-of-function.

Table 1. Some of the previous studies regarding the common polymorphisms in NOD2 gene and the associated diseases.

Number	SNPs	Mutation	Location	Population	Result	Infection (Disease)	Method	Ref
1	P268S	CCC > TCC	NBD domain	African Americans	Minor allele T is associated with a decreased risk of TB (Protective)	Tuberculosis	Sequencing of the coding regions of the NOD2 gene	[24]

Number	SNPs	Mutation	Location	Population	Result	Infection (Disease)	Method	Ref
	R702W	CGG > TGG [14]4	HD2 Exon 4		Minor allele T is associated with a decreased risk of TB(Protective)			
	A725G	GCT > GGT	HD2 Exon 4		the minor allele G increased the risk of TB			
2	R702W	CGG > TGG		South African	No association	Inflammatory bowel disease (CD & UC)	PCR of the Exons 4, 8 and 11- HEX-SSCP &RFLP	[25]
	A725G	GCT > GGT			Increased risk of TB			
	G908R	Rs2066845			No association			
	1007fs(insC3020)	L1007P rs5743293			No association			
3	rs3135499		Promoter	Han Chinese from Jiangsu Province	T genotype protective	Tuberculosis	TaqMan-based allelic discrimination system	[26]
	rs7194886		Promoter		Increased risk for T allele carriers			
	rs8057341		Promoter					
	rs9302752		Promoter		T genotype protective			

Number	SNPs	Mutation	Location	Population	Result	Infection (Disease)	Method	Ref
4	insC3020 R702W G908R	rs5743293 Rs2066844 Rs2066845		Sardinian population.	Significant Association (Increased the susceptibility)	CD & Mycobacterium avium subsp. paratuberculosis	PCR & sequencing	[27]
5	insC3020 R702W G908R	1007fs Rs2066844 rs2066845		northern Indian states	No mutation was observed in the patients and controls	TB and leprosy	PCR-RFLP confirmed by gene sequencing	[28]
6	R702W G908R			South African	No association	Tuberculosis	Tag Man platform genotyping	[29]
7	insC3020 P268S R587R R702W	C > T rs2066842 T > G rs1861759 C > T	Exon 4 Exon 4 Exon 4	Caucasian patients	No association	Sarcoidosis	Tag Man platform genotyping	[30]

Number	SNPs	Mutation	Location	Population	Result	Infection (Disease)	Method	Ref
		rs2066844						
	G908R	G > C rs2066845	Exon 8					
	insC3020	rs2066847	Exon 11					
8	P268S			Turkish population	Association with CD	Crohn's Disease and Ulcerative Colitis	PCR-RFLP	[31]
	M863V				No mutant was found			
	R702W	rs2066844 CGG > TGG			C allele is a risk factor			
9	G908R	rs2066845		Meta analysis	no associated	sarcoidosis	Meta-analysis	[32]
	insC3020	rs2066847			no associated			
	R587R	rs1861759			no associated			
10	C-159 T	rs2569190		Meta analysis	GG is common in TB	Tuberculosis	Meta-analysis	[33]
	A-1145G	rs2569191			T allele is a risk			

Number	SNPs	Mutation	Location	Population	Result	Infection (Disease)	Method	Ref
					factor in TB			
	IV	rs1861759			TG genotype is higher in TB			
		rs7194886			T allele is a risk factor of TB			
	R702W	rs2066844			CC genotype is a risk factor for TB			
	P 507 T/S	rs2066842 C > A/T			CC genotype is a risk factor for TB			

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11	-159C > T	-159C > T	promoter of CD14	Chinese	Higher risk increased promoter activity/increased sNOD2	spinal TB	Seq.	[34]
12	G-1619A	rs2915863	promoter of CD14	Han Chinese	Increased susceptibly/ increased sNOD2	tuberculosis	PCR and seq	[35]
	T-1359G	rs3138078						
	A-1145G	rs2569191						
	C-159T	rs2569190						

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Number	SNPs	Mutation	Location	Population	Result	Infection (Disease)	Method	Ref
13	C(-159)T G(-1145)A		promoter of CD14	Han Chinese	T allele is a RF G allele is a RF	tuberculosis	PCR-DNA sequencing	[36]
14	C(-159)T		promoter of CD14		increased level of serum soluble CD14	tuberculosis		[37]
15	C(-159)T		promoter of CD14	Mexico	increased Tb susceptibility/ increased level of serum soluble CD14		PCR-RFLP	[38]
16	C(-159)T		Promoter	Meta analysis	increased risk of TB		Meta-analysis	[39]
17	R426H	rs562225614 G > A	Exon 4	Case report	Early Onset Inflammatory Bowel Phenotype	IBD-Increased expression of inflammatory cytokines	Sequencing	[40]
18	N1010K	3030A > C	LRR domain Exon 12			CD	Sequencing	[41]

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