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Functions of the cytoplasmic RNA sensors RIG-I and MDA-5: Key regulators of innate immunity

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ABSTRACT

The innate immune system responds within minutes of infection to produce type I interferons and pro-inflammatory cytokines. Interferons induce the synthesis of cell proteins with antiviral activity, and also shape the adaptive immune response by priming T cells. Despite the discovery of interferons over 50 years ago, only recently have we begun to understand how cells sense the presence of a virus infection. Two families of pattern recognition receptors have been shown to distinguish unique molecules present in pathogens, such as bacterial and fungal cell wall components, viral RNA and DNA, and lipoproteins. The first family includes the membrane-bound toll-like receptors (TLRs). Studies of the signaling pathways that lead from pattern recognition to cytokine induction have revealed extensive and overlapping cascades that involve protein–protein interactions and phosphorylation, and culminate in activation of transcription proteins that control the transcription of genes encoding interferons and other cytokines. A second family of pattern recognition receptors has recently been identified, which comprises the cytoplasmic sensors of viral nucleic acids, including MDA-5, RIG-I, and LGP2. In this review we summarize the discovery of these cytoplasmic sensors, how they recognize nucleic acids, the signaling pathways leading to cytokine synthesis, and viral countermeasures that have evolved to antagonize the functions of these proteins. We also consider the function of these cytoplasmic sensors in apoptosis, development and differentiation, and diabetes.

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Abbreviations: Atg5, autophagy related 5 homolog (S. Cerevisiae); Atg12, autophagy related 12 homolog (S. Cerevisiae); CARD, caspase activation and recruitment domain; cDCs, conventional dendritic cells; CTD, C-terminal domain; DISH, differentiation induction subtraction hybridization; EMCV, encephalomyocarditis virus; ER, endoplasmic reticulum; FADD, Fas associated death domain; IFN, interferon; GMCSF, granulocyte macrophage colony stimulating factor; IKK, I kappa B kinase; IRF, interferon regulatory factor; IKKi, IkappaB-kinase-epsilon; IPS-1, interferon β promoter stimulator 1; LGP2, laboratory of genetics and physiology 2; MDA-5, melanoma differentiation associated gene-5; MEF, mouse embryo fibroblast; Mss4, mammalian suppressor of Sec4; NLRX1, NLR (nod-like receptor) family member X1; PAMP, pathogen-associated molecular pattern; pDCs, plasmacytoid dendritic cells; Poly(I:C), polyinosinic:polycytidylic acid; RA, retinoic acid; RIG-I, retinoic acid-inducible gene-I; RIP1, receptor-interacting serine-threonine kinase 1; RLH, RIG-like helicases; RNase L, ribonuclease L; RNF125, ring finger protein 125; SNP, single-nucleotide polymorphism; STING, stimulator of IFN genes; TBK1, TANK-binding kinase 1; TLR, toll-like receptor; TRADD, TNFR1-associated death domain protein; TRAF3, TNF receptor-associated factor 3; TRAP, translocon-associated protein; VSV, vesicular stomatitis virus.

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1. Introduction

The number of pathogens that we encounter daily is astronomical. Most are halted by an efficient defense system that has evolved over millions of years in the face of microbial infections. An important component of this defense arsenal is the innate immune system, which responds within minutes of infection to produce type I interferons (Isaacs & Lindenmann, 1957; Rubinstein et al., 1979; Friesen et al., 1981; Pestka & Baron, 1981). These antiviral proteins are produced by infected cells and lead to the synthesis of cell proteins, which halt viral replication, and also shape the adaptive immune response by priming T cells. Additionally, interferons also modulate a plethora of other important cellular functions including cell growth and differentiation, histocompatibility and tumor antigen expression, gene expression and anti-tumor effects (Fisher et al., 1983; Fisher & Grant, 1984; Giacomini et al., 1984; Grant et al., 1985; Greiner et al., 1984; Huang et al., 1999a,1999b; Jiang et al., 2000; Moulton et al., 1992; Sen & Sarkar, 2007; Su et al., 2008; Greiner et al., 1985; Greiner el al., 1987).

Despite the discovery of interferons over 50 years ago, only recently have we begun to understand how cells sense the presence of a virus infection and initiate cytokine synthesis. First insights came from the discovery of the toll-like receptors (TLRs) during a study of genes essential for the establishment of the dorsal–ventral axis in *Drosophila* (Nusslein-Volhard & Wieschaus, 1980). The TLRs were subsequently shown to be transmembrane proteins that distinguish unique molecules present in pathogens, such as bacterial and fungal cell wall components, viral RNA and DNA, and lipoproteins (reviewed in Medzhitov 2007). We now understand that pathogens are recognized as foreign by a family of host pattern recognition receptors. Examination of the signaling pathways that lead from pattern recognition to cytokine induction has revealed extensive and overlapping cascades that involve protein–protein interactions and phosphorylation. These culminate in activation of transcription proteins that control the transcription of genes encoding interferons and other cytokines (reviewed in Kawai & Akira 2008).

A second family of pattern recognition receptors has recently been identified, which comprises the cytoplasmic sensors of viral nucleic acids, including MDA-5, RIG-I, and LGP2. Here we review the discovery of these proteins, how they recognize nucleic acids, the signaling pathways leading to cytokine synthesis, and viral countermeasures that have evolved to antagonize the functions of these proteins. We also consider the role of these cytoplasmic sensors in apoptosis, development and differentiation, and diabetes.

2. Characterization of retinoic acid-inducible gene-I and melanoma differentiation associated gene-5

2.1. Identification of retinoic acid-inducible gene-I and melanoma differentiation associated gene-5

Retinoic acid-inducible gene-I (RIG-I, also known as DDX58) and melanoma differentiation associated gene-5 (MDA-5, also known as Helicard or IFIH1) are virus sensors expressed ubiquitously in the cytoplasm. RIG-I was initially identified as a gene induced in acute promyelocytic leukemia cells after treatment with retinoic acid (Sun, 1997). A few years later its role as an antiviral protein was reported. In fact, screening an expression cDNA library obtained from IFN-β-treated cells led to the isolation of RIG-I (Yoneyama et al., 2004). An IRF-reporter gene was introduced in L929 cells together with the cDNA library, and clones were selected for their ability to induce the promoter after transfection of poly (I:C) (Yoneyama et al., 2004). Recently a splice variant of RIG-I has been identified (Gack et al., 2008) which carries a deletion (residues 36–80) within the first caspase activation and recruitment domain (CARD) (Fig. 1).

MDA-5 was identified in a differentiation induction subtraction hybridization (DISH) screen (Huang et al., 1999a,1999b) that was designed to define genes regulated as a function of induction of terminal differentiation in human HO-1 melanoma cells (Jiang and Fisher, 1993). These DISH genes, many of which represented unique sequences not reported in the 1999 gene database, were named melanoma differentiation associated (*mda*) genes. *mda*-5 was one such novel upregulated DISH gene in HO-1 human melanoma cells induced to irreversibly lose growth potential and terminally differentiate by treatment with IFN-β and mezerein, a protein kinase C-activating compound (Kang et al., 2002).

2.2. Functional domains

RIG-I and MDA-5 are DExD/H RNA helicases, which possess two (CARD) domains at their amino terminus (Fig. 1). Together with LGP2, laboratory of genetics and physiology 2, they form the RIG-I-like receptors (RLRs) family. LGP2 possesses only the helicase domain and lacks the CARD domain (Takeuchi & Akira, 2008; Yoneyama & Fujita, 2008). RIG-I and MDA-5 share ~25% homology within the CARD domain regions and 40% within the helicase domain.

RIG-I and LGP2 have a similar repressor domain (RD) localized at the carboxyl terminus (Saito et al., 2007). Evidence suggests an interaction between the RIG-I repressor region (aa 723–925) and the CARD and helicase domains (helicase linker region aa 420–627). Interestingly, overexpression of the repressor domain blocks RIG-I-mediated signaling.

Recently, a C-terminal domain (CTD) has been described, which partly overlaps with the previously identified RD. The structure of the CTD of RIG-I has been determined by X-ray crystallography (Cui et al., 2008) and nuclear magnetic resonance (NMR) (Takahasi et al., 2008). It consists of a basic concave surface and the opposite side contains acidic residues. A positive cleft is the binding site for dsRNA and 5′-ppp RNA and also encompasses the biological activity of signal repression. The crystal structure of RD reveals a zinc-binding domain coordinated by four cysteines (C810, C813, C864 and C869) conserved also in MDA-5 and LGP2. Mutational studies have shown that the zinc coordination site is a key structural motif and is essential for RIG-I signaling.

The CARD is the effector domain, that transduces the signal when the molecule is activated. Overexpression of CARD induces constitutive signaling independent of viral infection (Yoneyama et al., 2004). It has also been shown that the CARDs negatively regulate the ATPase activity of RIG-I preventing its signaling in the absence of viral RNA (Gee et al., 2008).

Based on structural and functional studies, a model has been proposed for RIG-I activation. In the absence of its ligand, non-self RNA generated during viral infection, RIG-I is in an inactivated form. Binding of dsRNA or 5′-ppp RNA to the basic cleft in the CTD induces a