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RETINOIC ACID-INDUCIBLE GENE-I (RIG-I) AND DISEASES

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Abstract Retinoic acid-inducible gene-I (RIG-I) is a cytoplasmic protein regarded as putative RNA helicase. Immunohistochemical studies revealed high levels of RIG-I expression in epidermic cells in psoriasis, in macrophages in atherosclerotic lesions and in glomeruli of lupus nephritis. RIG-I expression was also demonstrated in macrophages and vascular endothelial cells in experimental animals with *Listeria* or Hanta virus infection. In vitro studies using cell cultures revealed the expression of RIG-I, in various cells including endothelial cells, macrophages and astroglial cells, in response to the stimulation with cytokines, lipopolysaccharide, double-stranded RNA, *Listeria monocytogenes*, etc. The studies employing the overexpression or RNA interference suggested that RIG-I is involved in the regulation of cytokine expression including CXCL10/IP-10 and CCL5/RANTES. These results suggest that RIG-I constitutes a part of the intracellular pathway for the regulation of inflammatory and immune responses.

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Key words: RIG-I; infectious diseases; inflammatory diseases

Cloning of RIG-I

All-trans-retinoic acid (ATRA) induces cellular differentiation and is clinically used for the treatment of acute promyelocytic leukemia. A research group from the Shanghai Institute of Hematology has tried to characterize the genes induced by ATRA in NB4 leukemia cell line using differential display technique. They isolated several ATRA-induced genes, retinoic acid-inducible gene (RIG)-A, B, C, etc; and submitted the nucleotide sequences of these genes to the Gene Bank. Thus RIG-I (not "one" but "ai") was registered in the Gene Bank in 1997 (accession number AF038963).

We have studied the regulatory mechanisms of expression and functional significance of RIG-I, particularly in vascular endothelial cells. Endothelial cells, subjected to proinflammatory stimuli, express various molecules including cytokines and adhesion molecules, which play critical roles in acute and chronic inflammation. In an attempt to isolate new genes induced by *Eschrichia coli* lipopolysaccharide (LPS), we cloned, using the subtraction technique, a couple of LPS-inducible genes in human umbilical vein endothelial cells (HUVEC), and one of the genes was identified as RIG-I[1)].

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Table 1. Expression of RIG-I in vivo

	Cell type	References
Human		
・Atherosclerotic	aortamacrophages	16
・Psoriasis	epidermic cells	15
・Lupus nephritis	mesangial area capillary loop	19
Experimental animals		
・*Listeria monocytogenes*	hepatic Kupffer cells splenic reticular cells	3
・Hanta virus infection	endothelial cells	6
・dsRNA injection	astrocytes	11

Table 2. Expression of RIG-I in vitro

Cell type	Stimuli	References
Leukemia cells	retinoic acid	-
Endothelial cells	LPS	1
	IFN-γ	12
	dsRNA	6
Vascular smooth muscle cells	IFN-γ	13
Bronchial epithelial cells	IFN-γ	14
	influenza A virus	9
Gingival fibroblasts	IL-1	20
	LPS	2
	dsRNA	2
Macrophages	IFN-γ	16
	Listeria	3
MCF-7 breast cancer cells	IFN-γ	17
Astrocytes	dsRNA	11
T24 bladder epithelial cells	IFN-γ	21
Keratinocytes	IFN-γ	15
	TNF-α	15

RIG-I protein has a DExH motif, and the DExH box protein family is known to function as RNA helicases. RNA helicases catalyze the unwinding of double-stranded RNA (dsRNA), and regulates RNA metabolism as transcription, processing, transport, translation and degradation. However, at this point, details of RIG-I function in RNA metabolism are largely unknown.

We developed a specific antibody against RIG-I and conducted immunohistochemical studies in order to investigate the potential roles of RIG-I in diseases. RIG-I was detected in affected tissues in several human diseases and experimental animal models (Table 1).

The regulatory mechanism for RIG-I expression was examined using cell culture systems in vitro. RIG-I expression was demonstrated in a wide variety of cell types in response to various stimuli (Table 2). These results suggest that RIG-I

controls inflammatory and immune responses and is involved in the pathogenesis of inflammatory and infectious diseases.

RIG-I and bacterial infection

We found the induction of RIG-I in vascular endothelial cells[1] and fibroblasts[2] in response to the stimulation with *E. coli* LPS[2]; and RIG-I may be involved in the pathological conditions elicited by infection with gram-negative bacteria and endotoxin. Also, immunohistochemical studies demonstrated the enhanced expression of RIG-I in hepatic Kupffer cells and splenic reticular cells of mice infected with *Listeria monocytogenes*, a gram-positive bacterium[3]. *L. monocytogenes* induced the expression of RIG-I in cultured RAW264.7 murine macrophage-like cells[3], as well.

RIG-I and viral infection

The recognition of pathogen molecular pattern distinguishable from host molecules is important in innate immunity, and Toll-like receptors (TLRs) are specialized in the pattern recognition of pathogen molecules. TLR3 recognizes dsRNA viruses, and the binding of dsRNA to TLR3 activates the signaling to induce anti-viral responses. Yoneyama et al[4,5] showed that RIG-I serves as a cytoplasmic receptor for dsRNA, independently on TLR3, and activates anti-viral responses including interferon (IFN) production.

The expression of RIG-I in vascular endothelial cells was upregulated in experimental animals inoculated with Hanta virus, which causes hemorrhagic fever with renal syndrome and encephalitis[6].

Polyinosinic-polycytidylic acid (poly IC) is authentic dsRNA, and treatment of cells with poly IC mimics RNA virus infection of the cells. Overexpression of RIG-I in BEAS-2B bronchial epithelial cells enhanced the poly IC-induced expression of chemokines CXCL-10/IP-10[7] and CXCL-1/growth related oncogene protein-α (GRO-α)[8]. RIG-I was demonstrated to be involved in the production of interleukin (IL) -28 and IL-29 by alveolar epithelial A549 cells and human dendritic cells in response to influenza A virus[9]. Thus RIG-I may play an important role in the host defense against viral infection in the airway.

CXCL-8/IL-8 is a potent chemoattractant for neutrophils and involved in inflammatory diseases. RIG-I was demonstrated to mediate the upregulation of IL-8 in hepatitis C virus (HCV) infection via transcriptional activation and mRNA stabilization[10]. Overexpression of RIG-I in gingival fibroblasts enhanced the IL-8 production induced by poly IC[2].

Viral infections in the central nervous system are often associated with severe syndrome. Astrocytes play an important role in maintaining the homeostasis and controlling the responses against various incidents including viral infection. Poly IC trearment of U373MG astrocytoma cells induced the expression of RIG-I; and RNA interference of RIG-I inhibited the poly IC-induced expression of CCL-5/RANTES[11], a chemokine that activates white blood cells including T lymphocytes.

These results suggest that RIG-I may be involved in anti-viral responses in various types of cells by regulating the expression of chemokines. One of the major functions of IFN-γ is its anti-viral activity. In our studies, IFN-γ induced RIG-I in vascular endothelial cells[12], vascular smooth muscle cells[13] and BEAS-2B bronchial epithelial cells[14]. Overexpression of RIG-I in BEAS-2B cells resulted in the up-regulation of signal transduced and activator 1 (STAT1), a key transcription factor in IFN-signaling[14]. Thus RIG-I may mediate anti-viral responses activated through the IFN-STAT1 pathway.

RIG-I and psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by infiltration of inflammatory

cells into the epidermis and altered keratinocyte differentiation. Psoriasis is currently considered a T-cell mediated 'Type-1' (Th1) autoimmune disease. IFN-γ is a major Th1 cytokine, and the IFN-γ-signaling pathways are implicated in the pathogenesis of this disease. We found the enhanced expression of RIG-I in keratinocytes in psoriasis skin lesions, and the expression of RIG-I was induced by IFN-γ in HaCaT keratinocyte cells in vitro[15]. RIG-I may be one of the key molecules in immune cell activation and in keratinocyte differentiation in psoriasis.

RIG-I and atherosclerosis

Formation of foam cells is regarded as a key process in the development of atherosclerosis. Inflammatory stimuli induce the expression of adhesion molecules on the endothelium, which leads to the adherence of circulating monocytes to endothelial surface and their migration into the intima of the arterial wall. The migrated monocytes differentiate into macrophages, which are then transformed into foam cells upon taking up modified lipoproteins. Therefore, the mechanisms for the recruitment and activation of white blood cells may play a role in atherogenesis; and atherosclerosis may be regarded, in one aspect, as a chronic inflammatory disease. We examined the expression of RIG-I, by immunohistochemical analysis, in atherosclerotic lesions in human aorta; and high levels of RIG-I immunoreactivity were detected in intimal macrophages in atherosclerotic lesions[16]. Inflammatory cytokines including IFN-γ play an important role in atherosclerosis. IFN-γ significantly induced RIG-I expression in THP-1 monocytic cells; particularly in the presence of phorbol 12-myristate 13-acetate, which promotes the differentiation of THP-1 cells into macrophage-like cells[16]. This suggests that RIG-I may be involved in differentiation and activation of macrophages, and play a role in the development of atherosclerosis.

RIG-I and malignancies

IFNs exert anti-tumorigenic effects, by regulating immune activities and/or affecting proliferation and differentiation of tumor cells, and IFN-inducible genes may contribute to the anti-tumorigenic activity of IFNs. We found the RIG-I induction, by IFN-γ, in MCF-7 breast cancer cells; and overexpression of RIG-I in MCF-7 cells resulted in the up-regulation of IFN-stimulated gene 15 (ISG15), a ubiquitin-like protein that has potential to mediate immunomodulatory effects of IFNs[17].

Epstein-Barr virus (EBV) is oncogenic; and EBV-encoded small RNAs (EBERs), abundant in latently EBV-infected cells, are implicated in the viral oncogenesis. RIG-I recognizes EBERs, and binding of EBERs to RIG-I initiates the signaling pathways leading to the induction of cellular genes such as IFNs, which are protective against viral infection[18].

As described above, ATRA induces the differentiation of leukemia cells; and RIG-I, as a gene induced by ATRA, may be involved in the differentiation of cells. Therefore, RIG-I may be a potential signaling molecule that serves as an anti-tumorigenic factor.

RIG-I and other diseases

RIG-I immunoreactivity was detected in the mesangial area and capillary loop in lupus nephritis; however, there was a trace, if any, of RIG-I expression in glomeruli of minimal change disease[19]. RIG-I was induced by IL-1, LPS or poly IC in cultured gingival fibroblasts[2,20]. Overexpression of RIG-I in fibroblasts enhanced the expression of cyclooxygenase-2 (COX-2), IL-8, IL-6 and IL-1β. IFN-γ up-regulated the RIG-I expression in T24 bladder epithelial cells in culture[21]. Overexpression of RIG-I in T24 cells also brought about the up-regulation of COX-2.

These findings suggest that RIG-I may be implicated in a variety of immune and

inflammatory diseases, by regulating the expression of inflammatory mediators. However, the details of its molecular function are still unknown and should be clarified in future studies.

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