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Review

Autophagy facilitates an IFN-γ response and signal transduction

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Abstract

Autophagy, that is directly triggered by invaded pathogens and indirectly triggered by IFN-γ, acts as a defense by mediating intracellular microbial recognition and clearance. In addition, autophagy contributes to inflammation by facilitating an IFN-γ response and signal transduction. For immune escape, downregulated autophagy may be a strategy used by microbes.

Keywords: Autophagy; IFN-γ; Inflammation; Signal transduction; Immune escape; Microbe

1. Introduction

Interferons (IFNs), including type I (IFN-α, IFN-β, IFN-ω, and IFNτ), type II (IFN-γ), and type III (IFN-λ), activate Janus activated kinases (JAKs) and signal transducer and activator of transcription (STAT), then trigger the expression of IFN-inducible anti-microbial and anticancer genes and proteins that regulate cell growth, proliferation, differentiation, cell death, and inflammation [1]. The rapid generation of IFNs accompanied by pro-inflammatory cytokines occurs through the activation of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors, C-type lectin receptors, and cytosolic nucleic acid sensors, including deoxyribonucleic acid (DNA)-dependent activator of IFN regulatory factors, absent in melanoma-2, ribonucleic acid (RNA) polymerase III, and leucine-rich repeat FLI-I interacting protein 1, in response to a variety of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns [2]. Autophagy, originally identified as an intracellular homeostatic mechanism for degrading aggregated proteins and damaged organelles through autolysosome formation, also has an important role in development, immune defense, programmed cell death, and neurodegeneration [3]. Notably, numerous studies have demonstrated that autophagy is essential for intracellular pathogen recognition [4], PRR activation followed by the IFN response and major histocompatibility complex (MHC) class II presentation [5,6], and IFN-γ signaling [7]. Treatment with IFN-γ triggers autophagy not only by enhancing intracellular microbial clearance and immune activation [8,9] but also by facilitating its downstream JAK2-STAT1 signaling and cellular inflammation [7]. Clinically, targeted therapies for human disease have involved treating and/or blocking IFNs given their important role in innate and adaptive immunity [10]. In this article, we review the potential role for autophagy in facilitating the IFN-γ response and signal transduction. A potential strategy for deregulating autophagy that is induced by microbial infection is also discussed.

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2. Biological roles of IFNs and IFN signaling

IFNs are pleiotropic cytokines with antiviral, anticancer, and immunomodulatory effects [1]. Generally, the immunoregulatory effects of IFNs include the following: (1) inducing MHC antigen presentation pathways; (2) developing T helper cell responses; (3) exhibiting anti-microbial properties; (4) promoting anticancer activities; (5) regulating leukocyte trafficking; and (6) facilitating lipopolysaccharide signaling. IFN-γ signaling pathways are crucial for controlling intracellular bacterial infections. $CD4^+$ T cells secrete IFN-γ to activate infected macrophages and induce microbicidal functions. IFN-γ induces intracellular bacterial killing via the production of nitric oxide and reactive oxygen species (ROS). These molecules play important roles in cellular signaling and in the regulation of cellular processes including inflammatory responses. $CD8^+$ T cells and natural killer (NK) cells also produce IFN-γ in response to a *Mycobacterium tuberculosis* infection which assists in the clearance of infected cells [11]. Although IFN-α/β is the first line of defense against a viral infection, IFN-γ is protective against some viral infections by upregulating proteins including protein kinase double strand RNA (dsRNA)-regulated (PKR), dsRNA-specific adenosine deaminase (ADAR1), and guanylate-binding protein (GBP) [1]. IFN-γ has direct anti-tumor effects; it inhibits cell proliferation, sensitizes tumors cells to apoptosis, upregulates MHC class I and II expression, and stimulates anti-tumor immune activity [10]. A recent report showed that $Ifng^{-/-}$ mice develop more tumors compared to wild type mice, demonstrating that IFN-γ plays a major role in mediating anti-tumor responses [12]. In addition, IFN-γ enhances the immunogenicity of tumor cells that are recognized and eliminated by the host defense by enhancing the expression of MHC antigen processing and presentation pathways, increasing T cell activation and NK cell killing, and increasing the expression of Fas-Fas ligand within tumors.

In terms of IFN signaling [1], the type I IFNs bind to the type I IFN receptors, IFNAR1 and IFNAR2. These two receptors are associated with JAK's tyrosine kinase 2 and JAK1, respectively. The type II IFN-γ binds a distinct type II IFN receptor that is composed of two subunits, IFNGR1 and IFNGR2, which are associated with JAK1 and JAK2, respectively. For JAK2-STAT1 activation, JAK2 is first autophosphorylated at its tyrosine residues (Y1007/1008) and then induces JAK1 transphosphorylation (Y1022/1023). The activation of JAK1 then phosphorylates IFNGR1 (Y440), which induces the recruitment and activation of STAT1 through JAK2-mediated phosphorylation (Y701). Activation of the JAKs also results in the tyrosine phosphorylation of STAT2 and STAT3. Both type I and type II IFNs also induce the formation of STAT1-STAT1 homodimers that translocate to the nucleus and bind GAS (on its IFN-γ-activated site) elements, thereby initiating the transcription of these genes [13]. In addition to JAK-STAT activation, p38 mitogen-activated protein kinase is activated and catalyzes the phosphorylation of Ser727 in both STAT1 and STAT3. Basically, serine/threonine kinase glycogen synthase kinase (GSK)-3β is activated to promote IFN-induced JAK-STAT signaling and inflammation [14–16]. Taken together, the regulation of JAK-STAT signaling is critical for IFN activity.

In regard to the feedback regulation of IFN signaling, three families of proteins, the SH2-containing phosphatases (SHP), the protein inhibitors of activated STATs, and the suppressors of cytokine signaling (SOCS), inhibit specific and distinct aspects of IFN signal transduction [17–19]. SHP2 dephosphorylates JAK1, JAK2, and IFNGR1. Additionally, STAT1 activation is downregulated by STAT1 dephosphorylation in the nucleus by SHP2. SOCS1 and SOCS3, induced by the JAK-STAT pathway, interfere with JAK activity by turning off signaling after ligand binding. SOCS1 binds to JAK's activated receptor and inhibits its kinase activity. Although IFNs independently induce negative feedback through JAK-STAT-SHP/SOCS signaling, the post-modification state of these proteins remains unclear. SHP2 becomes phosphorylated at Tyr542 and Tyr580 in its carboxy-terminus in response to growth factor receptor activation. However, the molecular mechanisms for SHP2 activation and expression remain unclear. Our previous results showed that inhibiting GSK-3β increased SHP2 phosphorylation at Tyr542 [15]. Activated SHP2 is able to downregulate the persistent activation of IFN-γ-induced JAK2-STAT1 signaling and decrease the activation of inflammatory mediators. We hypothesized that GSK-3β's involvement is important to IFN-γ signaling, especially in regard to SHP2-mediated feedback regulation.

3. IFN-based resistance to microbial infection

IFN-γ, produced by NK cells, NKT cells, T cells, and antigen presenting cells, is essential for anti-bacterial immunity, whereas IFN-α/β and IFN-λ are essential for antiviral immunity [20]. IFN-α/β is produced rapidly when viral factors, such as viral envelope glycoproteins cytosine-phosphate-guanosine motif DNA or dsRNA, interact with cellular PRRs, such as mannose receptors, TLRs, and cytosolic receptors. IFN-γ is induced by receptor-mediated stimulation, in response to early cytokines, or by stimulation from T cell receptors or NK cell receptors. Briefly, the antiviral actions of IFNs occur through the PKR-, ADAR1-, and GBP-regulated pathways. IFNs are able to upregulate their transcriptional expression [20,21]. PKR is a serine/threonine kinase activated by dsRNA, which inhibits viral protein synthesis by phosphorylating the α subunit of the eukaryotic translation initiation factor (eIF-2). The dsRNA-specific ADAR1 catalyzes the deamination of adenosine to form inosine on dsRNA substrates and thus may be responsible for the generation of "edited" viral mRNA. The GBP are guanine triphosphate hydrolases (GTPases) with antiviral properties that function by an unknown mechanism. When engaging in anti-bacterial activities, IFN-γ acts as a macrophage activator and promotes the innate defense mechanism of receptor-mediated phagocytosis and enhances microbial killing ability. To enhance the microbicidal ability, IFN-γ activates the nicotinamide adenine dinucleotide phosphate-dependent phagocyte oxidase system (respiratory burst) through the transcriptional induction of $gp91^{phox}$ and $p67^{phox}$, priming nitric oxide