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RIP1 modulates death receptor mediated apoptosis and autophagy in macrophages

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ABSTRACT

Macrophages are responsible for defending against diverse pathogens and play a crucial role in the innate immune system. Macrophage's lifespan is determined by homeostatic balance between survival and apoptosis. Here we report that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) triggers both apoptosis and autophagy in human U937 cells. Inhibition of autophagy facilitates TRAIL-induced apoptosis, suggesting that autophagy of macrophages protects against TRAIL-induced apoptosis. TRAIL treatment influences the expression of death receptors, indicating that TRAIL-induced apoptosis and autophagy are mediated by death receptors. RIP1 ubiquitination and expression regulate apoptosis and autophagy. Furthermore, expression and bioactivity of the p43/41-caspase-8 variant are critical to TRAIL-induced autophagy and apoptosis. Knockdown of RIP1 suppresses autophagy in macrophage. These data demonstrate that RIP1 is essential for the regulation of death receptor mediated autophagy and apoptosis. The results in this study contribute to understanding the regulation of autophagy and apoptosis in macrophages, and shed lights on death receptor-targeted therapy for cancer, inflammation and autoimmune diseases.

1. Introduction

Macrophages are widely distributed throughout body tissues and are essential components in the innate immune system. The cells exhibit effectors and antigen-presenting cell functions and are thus responsible for defending against diverse pathogens (Auffray et al., 2009). Macrophages originate from a common myeloid progenitor in the bone marrow. Normally, circulating monocytes are alive for a very short time before undergoing spontaneous apoptosis (Fahy et al., 1999). Upon stimulation, monocytes differentiate into macrophages, which have a longer lifespan (Wiktor-Jedrzejczak and

Abbreviations: c-FLIP, cellular FLICE inhibitory protein; DISC, death-inducing signaling complex; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LC3, microtubule-associated protein 1A/1B-light chain 3; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; rsTRAIL, recombinant soluble TRAIL; 3-MA, 3-methyladenine.

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Gordon, 1996). In the presence of certain stimuli, the apoptotic pathway of monocytes can be blocked, thus contributing to maintenance of the inflammatory response. As inflammation subsides, the cellular apoptosis program begins again and promotes regression of the immune response (Goyal et al., 2002; Savill and Fadok, 2000). A complex network of survival and death signal determines the fate of cells. Although monocytes are important defense components, the accumulation of monocytes may aggravate certain autoimmune diseases such as atherosclerosis, arthritis and multiple sclerosis (Linker et al., 2009). Understanding the interplay of survival and death signaling that coordinates the fate and function of macrophages has significant implications for research on cancer, inflammation and autoimmune diseases.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL, also known as apoptosis ligand 2, Apo2L) is a member of the TNF family, which has garnered extensive attention in the study of cancer, inflammation and autoimmune diseases. TRAIL is of particular interest in the development of cancer therapeutics as it is selectively cytotoxic to various tumor cells but has little or no toxic effect on most normal cells (Ashkenazi et al., 1999; Walczak et al., 1999; Wiley et al., 1995). Immune cell apoptosis is an important regulatory mechanism of innate immunity, which maintains homeostasis of the immune system and prevents autoimmune diseases. TRAIL is expressed in a variety of immune cells and plays an active role in various immune diseases, such as atherosclerosis, arthritis and multiple sclerosis (Cziupka et al., 2010; Martinez-Lostao et al., 2010; Yao et al., 2006).

Binding of homotrimeric TRAIL to its death receptor DR4 or DR5 drives receptor clustering into high molecular weight complexes, leading to the assembly of the death-inducing stimulating complex (DISC). The DISC is an aggregation of the intracellular death domain (DD) of the death receptor, the Fas-associated death domain (FADD), the apoptosis initiator caspase-8 or -10 and/or the caspase-8 or -10 inhibitor c-FLIP. In addition to the activation of caspases, TRAIL can also stimulate a cascade of intracellular reactions, such as activation of NF-κB, JNK, p38, MAPK and PKB/Akt. (Harper et al., 2001; Lin et al., 2000; Secchiero et al., 2003; Song and Lee, 2008; Varfolomeev et al., 2005; Zhang et al., 2003). In 'type I' cells, the DISC activates sufficient caspase-8 to stimulate effector caspase-3, -6 or -7, which results in apoptosis. However, in 'type II' cells, less active caspase-8 is generated by the DISC. Commitment of these cells to the apoptosis pathway requires further signal amplification by the intrinsic/mitochondrial pathway. In this case, an intracellular complex (known as complex II) composed of FADD, TRADD, caspase-8, caspase-10, RIP1, TRAF2 and IKK-γ (NEMO) is formed. The physiological role of these TRAIL-activated intracellular kinase cascades has yet to be fully elucidated (Varfolomeev et al., 2005).

RIP1 is an important regulatory protein in the DISC that can activate NF-κB and caspase-8 and generate reactive oxygen species (ROS). ROS are involved in the signal transduction of apoptosis, cell survival and programmed cell necrosis (Galluzzi and Kroemer, 2009; Kelliher et al., 1998; Lin et al., 1999). RIP1 function is modulated by ubiquitination and phosphorylation (Cho et al., 2009b; Ea et al., 2006). A previous report showed that in TNF-α-induced DISC, RIP1 and NEMO form a stable chain of linear ubiquitin. This complex is involved in determining cell survival, necrosis and apoptosis (Gerlach et al., 2011).

Cell death is a complex regulatory process. It is associated with at least three morphologically distinct processes: apoptosis, autophagic cell death (ACD) and necrosis. Autophagy or autophagocytosis is an evolutionarily conserved catabolic process involving the degradation of a cell's own components through the lysosomal machinery. As a protective mechanism to sustain cellular homeostasis, autophagy provides recycled resources for the cell by degrading long-lived proteins and senile cell organelles into small peptides or amino acids. In addition, autophagy can repress pathogenic infection and parasitization. It has been demonstrated that autophagy deficiency is related to various disorders including cancers, inflammation and cardiovascular diseases, implying the importance of autophagy in physiological and pathological processes (Kirkegaard et al., 2004; Levine and Kroemer, 2008). In general, autophagy is considered to be an adaptive response to external stimuli (such as hunger, infection) that can resist apoptosis and promote cell survival, but in excess, it may lead to autophagic cell death (Kondo et al., 2005; Maiuri et al., 2007).

The life span of macrophages is determined by the integration of survival and death signals (Doseff, 2004). Autophagy and apoptosis are vital intracellular signaling and metabolic pathways; however, the relationship between them and the regulatory mechanism in macrophages are unclear. In this study, we report that TRAIL induces both apoptosis and autophagy in human macrophage lymphoma U937 cells. Inhibition of autophagy significantly enhances death receptor mediated apoptosis. RIP1 expression and ubiquitination modification play an important role in the conversion of autophagy to apoptosis. These results not only contribute a detailed understanding of the molecular mechanisms controlling apoptosis and autophagy but also have significance for the clinical treatment of cancer, inflammation and autoimmune diseases.

2. Materials and methods

2.1. Cells and reagents

Human macrophage lymphoma cell line U937, human acute monocytic leukemia cell line THP-1 and mouse monocyte cell line RAW264.7 were purchased from American Type Culture Collection (Manassas, VA, USA). Cells were grown in RPMI 1640 medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (Hyclone), 100 U/ml penicillin and 100 g/ml streptomycin at 37 °C in a humidified atmosphere of a 5% CO_2. The pan-caspase inhibitor Z-VAD-fmk (FMK001) was purchased from R&D Systems, Inc. (Minneapolis, MN). Non-tagged recombinant soluble TRAIL protein (rsTRAIL, amino acid 95–281) was prepared as previously described by Guo et al. (Guo et al., 2005). Chloroquine diphosphate (C6628), 3-Methyladenine (M9281) was purchased from Sigma–Aldrich Co. (Taufkirchen, Germany) and LY294002, Wortmannin, IKK inhibitor-II and Wedelolactone from Merck Co. (NJ, USA).