Fas/FasL and Apoptosis/Non-apoptosis

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Abstract: The interaction of Fas and Fas ligand (FasL) is an important player in regulating apoptosis while Fas/FasL is also found to be involved into non-apoptosis regulation, which made it more attractive. To understand Fas/Fas ligand (FasL) and its function in the apoptosis and non-apoptosis regulation, a wide search was done by using internationally well-known databases and relevant papers were selected. Evidences supported that Fas/FasL signaling apoptotic pathway played a crucial role in the regulation of cell death and proliferation in various cell types. Numerous molecules were involved in the precise regulation of Fas/FasL mediated apoptosis through complicated signalling cascades. Nevertheless, the traditional apoptotic pathway, Fas/FasL signaling pathway, was also disclosed to function in non-apoptotic regulation in T cells via the interaction between T cell receptor and Fas/FasL and therefore Fas/FasL could be pivotal in maintaining the homeostasis of peripheral T cells and immune system.
***Keywords*:** Fas/CD95, Fas ligand/CD95L/CD178, apoptosis, non-apoptosis

1. Introduction

Apoptosis is the process of programmed cell death, which can ensure a homeostatic balance between the rate of cell formation and cell death physically. Therefore, apoptosis is crucial in the lifecycle of a cell from the point of development throughout the growth of an organism which contributes to the renewal of tissues and also the getting rid of sickness cells. Furthermore, apoptosis also provides an approach to target and kill cancer cells by immune cells. Fas/Fas ligand (FasL) is the widely known factor of apoptosis signalling pathway which regulates cell death. This review seeks to give an overview about the concept, function and regulation mechanism of Fas/FasL. Moreover, new research has shown non-apoptosis characteristics of Fas, which will also be touched in this review.

2. Methods

To obtain the published paper about Fas/FasL, a widly searching is made on Baidu Academics and also in databases on google and Bing including NCBI, Scopus, Web of Science by using the key words “Fas/CD95, FasL/CD95L/CD178, apoptosis, non-apoptosis”. The papers focusing on the mechanism of Fas/FasL leading to apoptosis and non-apoptosis are selected, for the final review.

3. Results

3.1 Fas/CD95

In 1989, two laboratories separately isolated two monoclonal antibodies with killing activity against a variety of human cells, and the antigens recognized by this antibody were defined as Fas and apoptosis antigen-1 (APO-1)[1,2], which were the same molecule, and were named CD95. It belongs to the nerve growth factor receptor (NGFR)/tumor necrosis factor receptor (TNER) group superfamily and is a cell apoptotic signalling receptor.

Fas/CD95 is a type I transmembrane glycoprotein with a molecular weight of 45 KD, containing 325 amino acids and a signal sequence at the N-terminal. The human FAS gene is located on the long arm of chromosome 10. Fas receptor (CD95) is a kind of death receptor (DR) expressed on the surface of many cells and activated a signaling pathway that regulates apoptosis. Fas mature protein includes three domains: extracellular, transmembrane and cytoplasmic and the extracellular domain consists of three cysteine-rich regions (CRD 1,2,3), which are specific sites for binding Fas ligands. The 49 amino acids at the N-terminal of Fas antigen are related to the trimerization of Fas antigen, which is the site where specific Fas ligands bind to Fas antigen and induce programmed cell death[3].The cytoplasmic domain contains a functional region that mediates apoptosis and is defined as the Death Domain (DD).

Fas exists in membrane molecular form (mFas) or soluble form (sFas). Fas can mediate apoptosis signals in sensitive cells that are mFas positive after being activated by FasL. sFas is a protein that is compiled after variable splicing of

Fas mRNA[4]. The naturally cleaved form of the sFasL fails to form oligomers with the Fas receptor, preventing it from triggering apoptosis. Moreover, sFasL is discovered to impede mFasL-mediated apoptosis by blocking its attachment to the Fas receptor, suggesting that mFas and sFasL have opposing roles that impact cell survival[5]. For instance, v-akt murine thymoma viral oncogene homolog(RAC-alpha serine/threonine-protein kinase (Akt1)), extracellular signal-regulated kinase, and JNK are activated when sFasL, which is secreted by a disintegrin and metalloproteinases, or MMPs, stimulates the proliferation of fibroblast-like synoviocytes in RA patients[6]. Hence, sFasL serves two purposes in terms of cell living and death.

Previous studies have shown that Fas can be widely expressed in the surface of immune cells[7] including thymus cells, activated T and B lymphocytes, NK cells, monocytes and parenchymal cells including liver and kidney, mediating cell apoptosis to maintain the stability of cell number.

3.2 Fas ligand

FasL, also named as CD178, CD95L, and Apo-1 ligand, is a homotrimeric membrane protein that belongs to the TNF superfamily. FasL is encoded by FASLG/Faslg gene in human/mouse with 5 exons both located on the chromosome 1. The homology of the FasL amino acid sequence between human and mouse and human and rat are 81% and 79.6%, respectively. FasL consists of a single transmembrane domain, an intracellular domain containing a proline-rich domain, and an extracellular domain[8]. The intracellular domain of FasL includes a hydrophobic amino acid domain but no signal sequence, and the C-terminal region (extracellular domain) is located outside the cell and it contains an oligomerization domain required for oligomerization, an important step to induce apoptosis. FasL has a spherical trimer structure and is a natural ligand for Fas antigens. Fas and FasL form the Fas system to mediate apoptosis[6]. FasL is mainly expressed on the surface of activated T lymphocytes, macrophages, and even non-immune cells such as mouse testis and retina of gld mice. The expression of FasL on activated T cells is triggered through stimulation by T cell receptor (TCR), costimulatory molecules, and cytokine receptors. The expression of FasL is controlled by multiple transcription factors, such as NF-κB, nuclear factor of activated T cells (NF-AT), transcription factors of the early growth response gene family, c-Myc, AP-1, secretory protein-1, and interferon regulatory factors[9,10].

Human/Mice without FAS/Fas expression could develop severe lymphatic accumulation disease (lymphoproliferation (lpr) phenotype), which is related to greatly accelerated aging of autoimmune related diseases. Genetic experiments have shown that a lack of the Faslg gene can cause similar changes, known as gld phenotype[11]. MRL lpr/lpr mice and MRL (generalized lymphoproliferative disease) gld/gld mice can develop lymphadenopathy and splenomegaly, producing large amounts of autoantibodies and eventually, lead to various autoimmune diseases such as arthritis.

3.3 Fas/FasL and cell apoptosis/non-apoptosis

In apoptotic pathway mediated by Fas/FasL, when FasL binds to Fas, it leads to the creation of Fas trimer. This activates the catalytic activity of Fas and initiates the recruitment of Fas-associated protein with death domain (FADD). After that, FADD enlists pro-caspase-8 and pro-caspase-10 to constitute the death-inducing signalling complex (DISC)[12](Figure 1). DISC is normally activated by several specific post-translational modifications, including palmitoylation and O-linked glycosylation. The DISC activates pro-caspase-8 and pro-caspase-10 in an autocatalytic manner, which enhances the death signal through the proteolysis of effector caspases such as caspases-3, caspase-6, and caspase-7. In contrast to FADD, TNF receptor-associated DD (TRADD), which is bound by TNF superfamily member 1A (TNFR1) and DR3, governs non-apoptotic functions and mainly mediates pro-inflammatory and immune-stimulatory activities by enlisting the DD-containing kinase receptor-interacting protein-1, and the E3 ubiquitin ligases TNF receptor-associating factor 2 (TRAF2) and cellular inhibitor of apoptosis proteins. (Figure 1).

It has been found that the Fas signal performs two roles, apoptosis and non-apoptosis, in various cells. In thymocytes, apoptosis is sufficiently initiated through the activation of caspases(Figure 1). By contrast, in B cells, the cleavage of BH3-interacting domain death agonist (Bid) mediated by caspase-8 is essential for apoptosis. Bid is a BH3-only protein which can promote the permeability of the mitochondrial outer membranes and the release of cytochrome c(Figure 1). Once cytochrome c is liberated from the mitochondria, it acts as a cofactor for the formation of a cytosolic caspase-activating complex called the apoptosome, which propagates the caspase activation cascade[14].

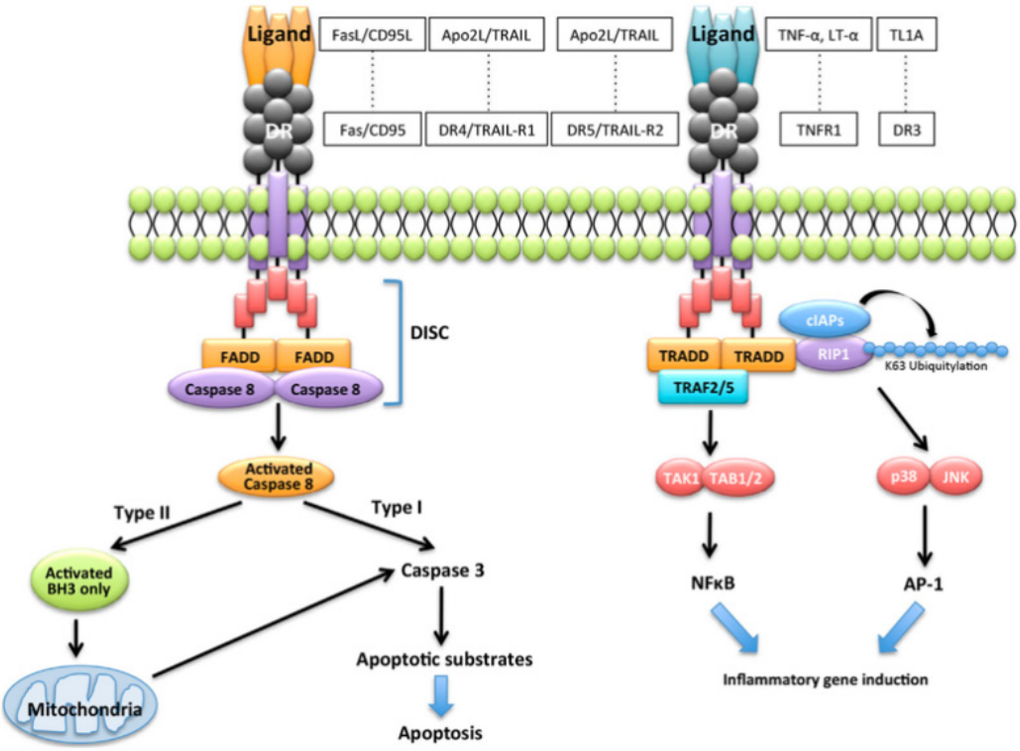

Figure 1. Apoptosis/non-apoptosis signal of Fas/FasL[13]

3.4 Fas-mediated T cell immune regulation

Three mechanisms maintain the homeostasis of peripheral T cells, including unresponsiveness, suppression by regulatory T cells, and activation-induced cell death (AICD)[15]. The AICD of peripheral T cells is controlled by the interactions between the T cell receptor (TCR) and Fas signalling in order to regulate the population of effector T cells. Additionally, AICD is brought about by the interaction between Fas and FasL, and activated T cells expressing Fas and FasL are eliminated either through self-destruction or by mutual interaction[16].

Antigen-stimulated effector T cells get activated to generate diverse inflammatory cytokines and growth factors during the immune responses. Nevertheless, overly activated effector T cells are detrimental to the immune system and need to be removed from the periphery. Even though costimulatory molecules like CD28 and programmed cell death protein-1 are widely known to manage the adjustment of TCR signaling, the system that reduces the activated T cells maintains peripheral tolerance. Hence, AICD brought about by Fas-mediated apoptosis has a significant role in the peripheral immune system[15].

The impairment of AICD leads to the beginning or advancement of autoimmunity. MRL-lpr/lpr mice have mutations in the gene encoding Fas and are widely utilized as a model for autoimmune disorders, like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), and autoimmune lymphoproliferation syndrome (ALPS)[17]. The Fas-mediated apoptosis of peripheral T cells, which demonstrates a crucial mechanism for maintaining immunological tolerance, is impaired in MRL-lpr/lpr mice. This also disrupts the elimination of overactivated or autoreactive T cells in the periphery. The elimination of T cells in the periphery is compromised in MRL-lpr/lpr mice, resulting in an increase in autoreactive or overactivated T cells and causing the induction of autoimmune lesions in multiple organs. Moreover, the mutations also occur in patients with ALPS[19].

In peripheral T cells, Fas activates both apoptotic and non-apoptotic pathways[20]. In the Fas signalling pathway, cellular caspase-8-like inhibitory protein (cFLIP) and TRAF2 are acted at the downstream in the cell, which contributes to the T-cell proliferation via Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation (Figure 2). The cFLIP N-terminal cleavage products p43-FLIP and p22-FLIP induce NF-κB activation by binding to the IκB kinase (IKK) complex[21] (Figure 2). Additionally, the overexpression of cFLIP hinders Fas-induced apoptosis of activated T cells. Fas signalling governs the homeostasis of peripheral T cells by balancing the equilibrium between proliferation and cell death in naive and memory T cell subsets[22]. Thus, the potent role of peripheral T cells might be stabilized through both results of FasL/Fas signalling.

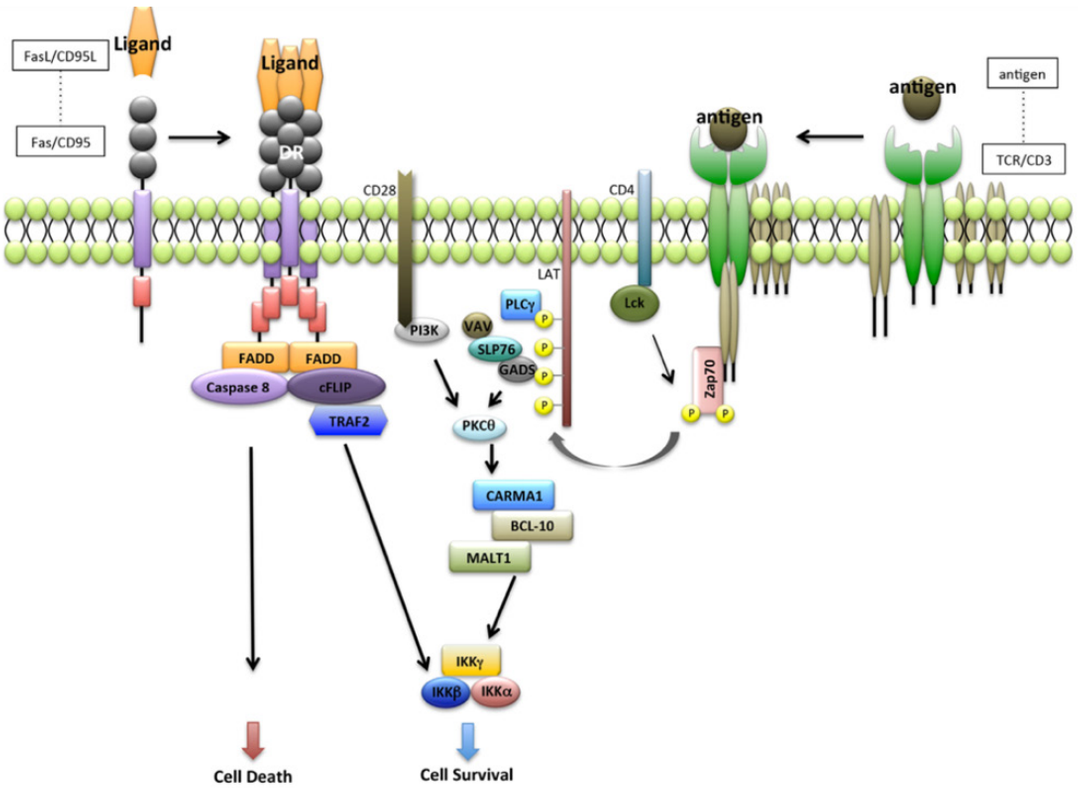

Figure 2. Apoptosis/non-apoptosis signal of Fas/FasL in peripheral T cells13

3.5 Regulators of Fas/FasL Signaling

Fas-mediated apoptosis is regulated by a variety of factors, including: 1) FLICE inhibitory protein: FLICE inhibitory proteins are similar in structure to FADD and caspase-8 and contain death domains, so they can compete with caspase-8 for binding sites in DISC to competitively inhibit Fas-mediated apoptosis. 2) c-myc: c-myc is a basic transcription factor of helix-loop-helix-zipper, and mutation experiments have proved that c-myc can promote apoptosis by regulating the transcription of target genes. C-myc can induce apoptosis by increasing cell sensitivity to Fas signals. 3) cytochrome c: cytochrome c is located in the inner mitochondrial membrane, and can be released into the cytoplasm after Fas induces apoptosis to amplify the effect of caspase-8. 4) Regulation of phosphorylation and dephosphorylation of protein kinases (such as Protein kinase C) and protein phosphatases (such as tyrosine phosphatase). 5) Regulation of protease in Fas pathway death signal includes transcriptional level regulation, post-translational modifications and the interaction of other regulators. 6) Bcl-2, Bcl-xl antagonize apoptosis, etc.

4. Conclusion

This review mainly shed light on one specific system, Fas/FasL signaling pathway, and its definition, function and regulatory mechanism in cell apoptosis/non-apoptosis. Evidences demonstrate that Fas/FasL signaling apoptotic pathway play an pivotal role in the regulation of cell death and proliferation in various cell types. Numerous molecules are proved to be involved in the precise regulation of Fas/FasL mediated apoptosis through complicated signalling cascades. Interestingly, the traditional apoptotic pathway, Fas/FasL signaling pathway, is also involved in non-apoptotic regulation in T cells, controlled by the interaction between T cell receptor and Fas/FasL signalling pathways.

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