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Molecular mechanisms of FasL-mediated ‘reverse-signaling’

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Summary

Effector lymphocytes, including NK and T cells, express FasL. Expression of Fas, the receptor for FasL in tumor cells, renders them susceptible to NK and T cell-mediated killing. The functional relevance of FasL in initiating death signals in tumor cells is well-characterized. However, the cytoplasmic interacting partners and the potential signaling pathways downstream of FasL are far from fully defined. FasL possesses an 81 amino acid long cytoplasmic tail with multiple unique recruitment motifs. We predict multiple interdependent signaling complexes form the core of the ‘reverse signaling’ downstream of FasL. A direct interaction between the proline-rich domain of FasL and the SH3 domain of PI(3)K-p85 α initiates the first pathway. This cascade helps FasL to link to PLC- γ 2 via PIP₃ or the Akt-dependent activation of mTOR complexes. Independently, a GRB2/GADs-binding PXXP cytoplasmic motif of FasL can initiate a Ras-GTP-dependent PAK1→C-Raf→MEK1/2→ERK1/2 activation. FasL can recruit Fyn via the proline-rich domain leading to the recruitment of ADAP. Through its ability to directly interact with Carma1 and TAK1, ADAP initiates the formation of the Carma1/Bcl10/Malt1-based CBM signalosome that is primarily responsible for inflammatory cytokine production. Here, we explore the conserved cytoplasmic domains of FasL, the potential signaling molecules that interact, and the functional downstream consequences within the effector lymphocytes to define the FasL-mediated ‘reverse signaling’.

Keywords

Fas; FasL; reverse signaling; cell death; signaling molecules

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Conflict of interest

The authors state no conflict of interest.

1. Introduction

Fas ligand (FasL, APO-1L, CD178) is a membrane-bound, 40 kDa, type II protein that recognizes Fas (Nagata and Golstein, 1995). Both FasL and Fas belong to the TNF superfamily (Suda et al., 1993). The cytoplasmic domain of FasL is highly conserved across species and contains the most extended amino acid sequence (81 residues) of the 19 known TNF family members (Bodmer et al., 2002). Activated immune cells, such as T, B, DC, and NK cells express FasL (Yamada et al., 2017). Resting cells express only minimal levels of FasL; however, stimulation with IL-2 and IL-12 or IL-2, IL-12 and IL-18, significantly enhances the levels of FasL in lymphocytes (Malarkannan, unpublished) (Refaeli et al., 1998). One possible mechanism by which these cytokines can augment the expression of FasL is by downregulating the expression of MMP3 and MMP7, responsible for cleavage of the ectodomain of FasL from the cell surface as shown for IL-12 (Gorelik et al., 2004). FasL-expressing immune cells recognize the Fas receptor on a target cell, which results in the initiation of a complex apoptotic pathway in target cells (Montel et al., 1995; Nagata and Golstein, 1995). Downstream signaling that regulates apoptotic or non-apoptotic functions of Fas has been well-defined (Figure 1A). A conformational change in the extracellular domain of FasL in *gld/gld* mice due to a change in Phe²⁷³→Leu²⁷³ severely affects its ability to interact with the Fas receptor (Roths et al., 1984). FasL forms a homo-trimeric structure on the plasma membrane and possesses an extraordinarily long cytoplasmic tail (Holler et al., 2003). FasL mediates apoptotic cell death of targets (Montel et al., 1995; Shrestha and Diamond, 2007).

FasL exists in the cell membrane as a homo-trimeric or hexameric complex, exemplifying its potential to regulate multiple signaling pathways concurrently, which interacts with a homo-trimeric Fas on the surface of the target cell (Nagata and Suda, 1995). A unique aspect of Fas and FasL protein is the generation of their cleaved ectodomains from the membrane (Hohlbaum et al., 2000). The bound and soluble FasL mediate opposing functions (Gregory-Ksander et al., 2019; Gregory et al., 2011). The presence of soluble FasL in divergent pathological conditions leads to suppression of inflammatory responses and cell death primarily because of blocking the interactions between membrane-bound Fas-FasL interactions (Gregory et al., 2007; Krishnan et al., 2016; Matsumoto et al., 2015). Importantly, cell proliferation but cell death is augmented by the interaction of soluble FasL to membrane-bound Fas (Herrero et al., 2011). Both soluble FasL and Fas were elevated in patients with autoimmune NMDAR-encephalitis (Ding et al., 2018). Compared to soluble FasL, soluble Fas protected hepatocytes from Fas-dependent apoptosis but not a pathological effect of Fas-mediated TNF- α produced by the Kupffer cells in the liver (Matsuki et al., 2002). These findings reveal that the soluble FasL can be generated *in vivo* and can mediate a distinct set of biological functions from that of the membrane-bound. Irrespective of these clinically-relevant outcomes, the signaling events downstream of membrane-bound Fas or FasL that are initiated following the binding of their respective soluble partners are not known. FasL-independent activation of Fas has been described by using small molecules such as camptothecin (Shao et al., 2001) or doxorubicin-induced suppression of ADAM10 (W. H. Liu and Chang, 2011). On the contrary, FasL has been shown to interact with other partners such as secreted DcR3 (W. Liu et al., 2016), which is highly elevated in patients

with various cancers that may aid in the immune evasion of tumor cells (Roth et al., 2001; Takahama et al., 2002). Whether the binding of cell-free secreted DcR3 to FasL can initiate signaling is not yet known.

The Fas-FasL interaction is one of the most-studied receptor-ligand pairs across the multidisciplinary area. Forward signaling events downstream of Fas, including FADD, caspase 3, caspase 8, RIP1, and Ask1 (Figure 1A), are involved in apoptotic cell death (Kreuz et al., 2004). Intriguingly, FasL does not require any component of its cytoplasmic tail for mediating target cell death (Luckerath et al., 2011). However, using its cytoplasmic tail, FasL is known to enable other non-apoptotic effector functions, which includes a 'reverse-signaling'-mediated costimulation of T cells (Sun et al., 2007; I. Suzuki et al., 2000). However, the biological relevance of FasL-mediated 'reverse signaling' is yet to be fully understood. FasL can function as a co-stimulatory molecule in T cells (I. Suzuki and Fink, 1998). Also, FasL promotes the proliferation of peripheral CD4⁺ and CD8⁺ T cells (I. Suzuki and Fink, 2000; I. Suzuki et al., 2000). Most importantly, FasL is required for the positive selection and maturation of MHC-Class I- and Class-II-restricted T cells with moderate TCR affinities (Boursalian and Fink, 2003).

The non-lytic mechanism utilized by FasL on NK cells to kill target cells required multiple conjugations that resulted in slower kinetics (Zhu et al., 2016). However, the granzyme B-dependent lytic method was effective with one prolonged conjugation with faster dynamics. In addition, the trafficking of FasL-containing vesicles to the plasma membrane and the FasL-mediated target cell lysis was inhibited by Ly49-MHC Class I interactions (Wallin et al., 2003). Irrespective of its ability to mediate costimulation, the function of FasL as an independent activation receptor has not been established. The presence of multiple protein-interacting motifs in its cytoplasmic tail strongly suggests the ability of FasL to be a capable activation receptor (Ghadimi et al., 2002; Sun et al., 2006; Thornhill et al., 2007). These observations demonstrate that the downstream signaling of FasL is distinct and complex. The clues to understanding these complexities are embedded in the cytoplasmic tail of FasL. In this review, we analyze the potential binding motifs present in the cytoplasmic tail of FasL, predict the presence of multiple activation pathways, and develop a molecular blueprint of activation cascades downstream of FasL.

2. Recruitment domains in the FasL cytoplasmic tail

The FasL cytoplasmic tail is 81 amino acid long and consists of multiple motifs capable of recruiting a variety of signaling molecules (Figure 1B) (Ghadimi et al., 2002; Hane et al., 1995; Thornhill et al., 2007; Wenzel et al., 2001). The homo-trimeric complexes of FasL add further complexity to the downstream signaling pathways. The motifs in the cytoplasmic tail of FasL include two casein kinase 1 (CK1) binding motifs [SSA(T/S)] that could potentially be involved in GSK3 β phosphorylation and thereby β -catenin/Lymphoid enhancer-binding Factor-1 (LEF-1)/T cell factor-1 (TCF-1) activation downstream of FasL (Watts and DeBenedette, 1999). A proline-rich domain (PRD), [(R/K)XXPXXP], present in the proximal N-terminal region of FasL, functions as a binding site for Class I Src homology 3 (SH3)-containing proteins such as Fyn, ITK, and PI(3)K-p85 α (Hane et al., 1995; Voss et al., 2009). FasL also contains a C-terminal Class II SH3-binding site [PXXPX(R/K)] that

can interact with the SH3 domains of adaptor molecules Grb2, Gads, and Grap (Thornhill et al., 2008). There are nine Gads-interacting motifs (PXXP) distributed throughout the cytoplasmic tail of FasL (Wenzel et al., 2001). Additionally, other SH3-containing proteins such as Hck, Lyn, Nck, and sorting nexin family members SNX9, SNX18 and SNX33 are known to interact with the PRD domain of FasL (Voss et al., 2009; Wenzel et al., 2001). FasL also contains a WW domain-binding (XPPPXXPX) motif that interacts with Dystrophin, FBP11, and FE65 (Figure 1C, D) (Wenzel et al., 2001).

FasL cytoplasmic tail also contains one tyrosine, three threonine, and eight serine residues, and the latter is phosphorylated upon CD3 and FasL-mediated activation in T cells (Sun et al., 2007). The presence of such a high molecular complexity of the FasL cytoplasmic tail strengthens the notion that multiple signaling pathways are operative downstream of FasL. Based on the earlier studies and the divergent motifs present in the cytoplasmic tail of FasL, multiple interdependent signaling complexes could be responsible for the ‘reverse signaling’ downstream of FasL. The regulatory PI(3)K-p85 α subunit interacts with the proline-rich domain (RPPPPPP) of FasL via its SH3 domain and recruits its catalytic subunit PI(3)K-p110 δ (Figure 1D). This helps FasL to link to PLC- γ 2 via PIP₃. FasL can recruit Fyn via the same proline-rich domain (RPPPPPP), leading to the recruitment of ADAP by Fyn. ADAP recruits Carma1 and TAK1 to two of its interacting domains and initiates the formation of the Carma1/Bcl10/Malt1-based CBM signalosome. CBM activates the NF- κ B and AP1 (c-Jun/c-Fos) for the transcription of hundreds of genes, including the ones encoding inflammatory cytokines and chemokines (Rajasekaran et al., 2011b; Rajasekaran et al., 2013). Thus, the binding motifs present in the cytoplasmic tail of FasL can recruit a multitude of intracellular partners to convert the extracellular stimuli into a complex matrix of signaling cascades.

3. Fyn-Lck-PI(3)Kp85 α /p110 δ -ITK-PLC- γ pathway

The first pathway involves the activation of PI(3)K that links to PLC- γ , mTORC1, and mTORC2 and multiple other downstream signaling cascades (Figure 2). Membrane-proximal signaling events downstream of FasL involve Fyn and Lck. The FasL possesses binding sites for Class I Src homology 3 (SH3)-containing proteins such as Fyn. Transfection studies have shown that the GST-SH3 domain of Fyn can co-immunoprecipitates with FasL and a proline-rich domain, RPPPPPP of the FasL cytoplasmic tail was also able to recruit Fyn (Hane et al., 1995; Wenzel et al., 2001). Thus, both Fyn and Lck constitute the earliest membrane-proximal signal transducers of FasL. Therefore, we propose that Fyn and Lck can link FasL to the PI(3)K pathway. Fyn and Lck have been shown to increase the activity of p85 α /p110 dimer *in vitro* (da Silva et al., 1997). Class I PI(3)Ks generate secondary lipid messengers that regulate a wide array of intracellular signaling pathways in numerous cell types (Vanhaesebroeck et al., 2010). FasL can directly interact with PI(3)K-p85 α (Sun et al., 2007; Wenzel et al., 2001). Studies have indicated that activation via FasL (‘reverse signaling’) can mediate costimulation in T cells (Sun et al., 2006; I. Suzuki and Fink, 1998). Signaling molecules such as PI(3)K and Fyn are recruited to the FasL cytoplasmic tail after activation in T cells (Sun et al., 2007). Mammalian cells contain several isoforms of regulatory p85 (p85 α , p55 α , p50 α , p85 β , and p55 γ) and catalytic p110 (p110 α , p110 β , p110 γ and p110 δ) subunits, which exert distinct functions

(Vanhaesebroeck et al., 2001). For example, mice lacking the p85 α regulatory or p110 δ catalytic subunit show severely impaired B and T cell development and function (Fruman et al., 1999; H. Suzuki et al., 1999). The deletion of individual catalytic or regulatory subunits results in altered expression of other subunits (Vanhaesebroeck et al., 2005; Vanhaesebroeck et al., 2004). PI(3)K-p85 α interacts with PI(3)K-p110 δ , these observations support the critical role of PI(3)K p85 α /p110 δ complex in FasL-mediated activation.

Other signaling components that FasL cytoplasmic complex can recruit include ITK that links to PLC- γ 2 and its phosphorylation. It is tempting to suggest that the PRD region of FasL might directly recruit PLC- γ 1 in T cells or PLC- γ 2 in NK cells via the SH3 domain that is highly conserved in both PLC- γ 1 and PLC- γ 2 (Yablonski et al., 2001). However, no direct evidence exists. The SH2/SH3 domain-containing adapter NCK1 and the Tec family kinase ITK are the strong candidates to function as linkers between the FasL cytoplasmic tail and PLC- γ proteins. ITK uses its SH3 and SH2 domains to associate with the PRD region (amino acids 184 to 195) and the phosphorylated tyrosine (Y145) of SLP76, respectively (Bunnell et al., 2000; Grasis et al., 2010). This interaction results in the recruitment of the adaptor proteins ADAP and LAT, regulators of PLC- γ 2 phosphorylation (Bunnell et al., 2000; Ching et al., 2000; Peterson et al., 2001). In addition to ITK, LAT can also associate with FasL following activation. Gads and Grap but not Grb2 co-immunoprecipitates with FasL, which explains the association of LAT with the FasL (Figure 2, Malarkannan, unpublished). NCK1 uses its SH3 domain to interact with the PRD domain of FasL. Importantly, similar to FasL, NCK1 also localizes to the vesicular membranes of secretory lysosomes that transports the granzymes and perforin to the immunological synapse formed between the effector and target lymphocytes (Lettau et al., 2006). Thus, the possibility of NCK1 being the linker between FasL and PLC- γ is higher. These possibilities predict that FasL can function as an independent activation receptor in T and NK cells.

The second aspect of this pathway involves the cellular machinery essential for protein translation and transcriptional regulation. The PI(3)K-PDK1-Akt pathway is also responsible for the activation of the two mTOR complexes, which regulates several essential cellular processes. The activation of mTORC1 downstream of receptor complexes depends on the heterotrimeric Tuberous Sclerosis Complex 1 (TSC1), TSC2, and TBC1D7 (Dibble et al., 2012). TSC is a GTPase activating protein (GAP) that inhibits GTPase Rhab, which is required for the activation of mTORC1 (Inoki et al., 2003; Long et al., 2005; Tee et al., 2003). Both PI(3)K-PDK1-Akt and MAPK pathways promote the phosphorylation of TSC2 and inhibit the function of TSC (Inoki et al., 2002; Ma et al., 2005; Manning et al., 2002; Roux et al., 2004). mTORC1 promotes cellular growth and proliferation by activating 4EBPs and S6K1, which initiates and augments protein, lipid, and nuclear acids synthesis. Similarly, PI(3)K regulates the activation of mTORC2 (Sarbasov et al., 2005). Under steady-state, the kinase activity of mTOR is inhibited by the pleckstrin homology (PH) domain of mSin1. PI(3)K through its generation of PtdIns(3,4,5)P3 releases the inhibition of the kinase domain of mTOR (P. Liu et al., 2015). Also, downstream of PI(3)K, Akt phosphorylates mSin1 at Thr86 site, which results in the activation of mTORC2 (Yang et al., 2015). Thus, the activation of Fyn-PI(3)K by FasL can lead to mTORC1 and mTORC2-dependent transcriptional and transcriptomic alterations, metabolic reprogramming, and cell proliferation in T and NK cells. A link between FasL and mTOR-mediated signaling may

provide a mechanistic basis for several autoimmune disorders, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), and autoimmune lymphoproliferation syndrome (ALPS). Mutations in FasL that lead to these autoimmune diseases are provided as the basis for the lack of activation-induced cell death (AICD) of autoreactive T cells (Ishimaru et al., 2001). However, a potential overactivation via FasL that leads to impaired transcriptome and metabolic reprogramming is yet to be explored. Other potential examples include FasL-driven insulinitis in a non-obese diabetic mouse model of type 1 diabetes (Saxena et al., 2017). A detailed analysis signaling downstream of FasL in effector lymphocytes in these could provide mechanistic insights into the pathology of these autoimmune diseases.

4. Grb2-Gads-Ras-Raf-MAPK pathway

The cytoplasmic tail of FasL possesses motifs that can recruit and initiate the activation of MAPKs. The C-terminal Class II SH3-binding site [PXXPX(R/K)] of FasL can interact with the SH3 domains of adaptor molecules Grb2, Gads, and Grap (Thornhill et al., 2008). There are nine Gads-interacting motifs (PXXP) present in the FasL (Wenzel et al., 2001). Thus, a potential activation-dependent conformational change in the cytoplasmic tail of FasL can recruit GADs and GRB2 that lead to the recruitment and activation of the molecular switch Ras. There are multiple mechanisms that Ras-GTP can employ to link to the MAPK activation. First, activated Ras-GTP functions as a major linker between PI(3)K and MAPK activation (Fukushima et al., 2019). Ras can achieve this by acting as a regulator of the transition between $PIP_3 \leftrightarrow PIP_2$ via the PTEN/SHIP bistable system mediated by PI(3)K. Thus, Ras can function as a rheostat of PI(3)K activation and regulate the downstream PLC- γ -DAG-PKC-RalGDS-dependent activation of MAPKs. Secondly, Ras can directly interact with the Ras-binding domain (RBD) and thereby activating any isoform of the catalytic PI(3)K-p110 subunit independent of the regulatory PI(3)K-p85 α , which links to the PLC- γ by producing its substrate phosphatidylinositol-4,5-bisphosphate (PIP_2). This places Ras upstream of PI(3)K activation. Thirdly, GADs and GRB2 can associate with FasL and initiate the Ras-GTP-dependent $PAK1 \rightarrow C\text{-Raf} \rightarrow MEK1/2 \rightarrow ERK1/2$ activation. This MAPK pathway can be coordinated by scaffold proteins such as IQGAP1 (Abel et al., 2015; Awasthi et al., 2010; Malarkannan et al., 2012) or KSR1 (Basu et al., 2017).

Independent of the above possibilities, the LAT can function as a surrogate adaptor molecule to the cytoplasmic tail of FasL. Association of LAT with FasL could be mediated through Grb2, Gads, or Grap. The cytoplasmic tail of FasL contains one Grb2-Class II SH3-binding motif [PXXPX(R/K)] and one Grap-binding motif. Earlier studies have found that Grb2 associates and regulates FasL expression in Schwann cells (Thornhill et al., 2008). Also, Gads can constitutively associate with SLP-76 through its SH3 domain, and, after TCR activation, the SH2 domain of Gads associates with LAT (Asada et al., 1999; Law et al., 1999). This demonstrates that other members of the Grb2 family could potentially play a role in FasL-mediated activation. NKG2D-mediated signaling in NK or T cells requires DAP10-Grb2 interaction via the YINM motif of DAP10 (Upshaw et al., 2006). The FasL cytoplasmic tail does not have a YINM motif; instead, it contains 9 PXXP motifs, known to interact with Gads. Therefore, it is likely that FasL directly interacts with Grb2 or Gads;

however, further studies are required to determine the functional relevance of LAT association to FasL.

5. Fyn-ADAP-Carma1-Bcl10-Malt1 signalosome

The second pathway involves the adaptor protein, ADAP (also known as Fyb). Fyn binds to ADAP with high affinity (Figure 3) (da Silva et al., 1997; J. Liu et al., 1998). ADAP can recruit Carma1 via its C-terminal SH3/MAGUK region and thereby play an essential role in forming the CBM signalosome (Medeiros et al., 2007). Also, ADAP can interact with TAK1 directly [47]. Other members of the Tec family that use PIP₃ as lipid anchor are ITK, Rlk, and Tec that are mainly expressed in NK and T cells. We propose that following FasL-mediated activation, ADAP recruits ITK or SLP76. Earlier studies have demonstrated a direct association of ITK with the FasL cytoplasmic tail (Wenzel et al., 2001). Furthermore, FasL expression is reduced in *ITK*^{-/-} mice (Miller and Berg, 2002), suggesting that ITK is required for optimal FasL signaling. Thus, in the second major signaling pathway, Fyn could facilitate the formation of ADAP/ITK/SLP76 complex.

Fyn-ADAP axis regulates the production of inflammatory cytokines and chemokines, an essential effector function of NK and T cells (Medeiros et al., 2007; Rajasekaran et al., 2011a; Rajasekaran et al., 2013; Srivastava et al., 2010). The SH2 domain of Fyn recruits ADAP using a unique pYDGI motif with the amino acid sequence located between 625-628 residues of ADAP (Gerbec et al., 2015). Following the recruitment to the membrane, the Fyn/ADAP complex engages the C-terminal region of Carma1 and the eventual association to Bcl10, and Malt1, resulting in the Carma1-Bcl10-Malt1 (CBM) signalosome (Figure 3). CBM signalosome is essential for the activation and nuclear translocation of NF- κ B and AP1 that is, in turn, responsible for the inflammatory cytokine and chemokine production (Srivastava et al., 2010). Thus, the ability of FasL to recruit Fyn and interact with ADAP through its SH2 domain in initiating the ADAP-CBM signalosome to generate inflammatory cytokines in NK or T cells underscores its physiological importance. Intriguingly, the absence of ADAP exclusively reduced the production of inflammatory factors including IFN- γ , TNF- α , GM-CSF, CCL3, CCL4, and CCL5 and did not reduce the cytotoxic potentials of effector lymphocytes (Rajasekaran et al., 2013; Thakar et al., 2019). In contrast absence of Fyn reciprocally changed both cytokine production and cytotoxicity, indicating that downstream of activation or inhibitory receptors, Fyn initiates numerous signaling and can result in divergent outcomes. To date, over 100 SH2 domain-containing proteins have been identified (Kesti et al., 2007; Machida et al., 2007). Thus, FasL functioning as an independent activation receptor in T or NK cells can result in optimal inflammatory responses during an immune response and potentially cause chronic or acute inflammation during a pathological condition.

ITK links to PLC γ 2 phosphorylation and the downstream signaling molecules such as CARMA1 and TAK1 in NK cells. Previous studies have shown that Carma1 (Rajasekaran et al., 2011a) and Bcl10 (Malarkannan et al., 2007) play critical roles in NKG2D-mediated activation in NK cells. Transforming growth factor β (TGF- β)-activated kinase (TAK1) is a member of the mitogen-activated kinase kinase kinase (MAPKKK) family and is activated by a variety of stimuli (Ninomiya-Tsuji et al., 1999). Following activation, endogenous

TAK1 is recruited to the TRAF6 complex and stimulates both the INK and NF- κ B pathways (Morlon et al., 2005), regulating cytokine and chemokine production. Other studies have indicated that TAK1 can be activated via TRAF2 or TRAF6, independent of Carma1 (Baud et al., 1999). TAK1 regulates the NF- κ B pathway through its interaction with TRAF6 and by phosphorylating the NF- κ B-inducing kinase (NIK) (Schulze-Luehrmann and Ghosh, 2006). Thus, TAK1 can link between Carma1 and JNK1/2 (c-Jun/AP1) or NF- κ B in the reverse signaling mediated by FasL. TAK1 regulates the activation of p38 and JNK1/2 through the phosphorylation of MKK3/6 (Greenblatt et al., 2010) and MKK4 (Geuking et al., 2009), respectively. Activation of c-Jun/AP1 is regulated by JNK1/2 (Ventura et al., 2003). The CBM signalosome is the major upstream activation of NF- κ B and AP1 transcription factors that are obligatory for the transcription of genes encoding pro-inflammatory factors (Rajasekaran et al., 2011a; Rajasekaran et al., 2013; Rajasekaran et al., 2016). Thus, the ability of FasL to initiate a pro-inflammatory response under a pathological condition should be explored.

6. Summary and future outlook

We identify multiple interdependent signaling cascades that constitute Fyn/Lck/PI(3)K-p85 α -p110 δ /GADs/ITK/SLP76/ADAP/LAT/PLC- γ 2 and Fyn/ADAP/CBM signalosome. These predictions set a critical stage to define the independent or co-stimulatory effector functions mediated by FasL in effector lymphocytes that recognize and eliminate malignant cells. The evolutionarily conserved 81 amino acid long cytoplasmic tail of FasL with multiple interactive motifs is an exceptional challenge. Defining the molecular basis of the 'reverse signaling' by FasL is of high clinical relevance. Deciphering the intricacies of molecular interactions regulated by the homo-trimeric FasL molecule will provide opportunities in formulating improved and augmented cellular therapies targeting relapsing and resistant tumor cells. Determining how the distinct motifs in the cytoplasmic tail of FasL organizes a complex signaling events can benefit newer and better therapeutic approaches.

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References

- Abel AM, Schuldt KM, Rajasekaran K, Hwang D, Riese MJ, Rao S, Thakar MS, Malarkannan S, 2015 IQGAP1: insights into the function of a molecular puppeteer. *Mol Immunol* 65, 336–349. [PubMed: 25733387]
- Asada H, Ishii N, Sasaki Y, Endo K, Kasai H, Tanaka N, Takeshita T, Tsuchiya S, Konno T, Sugamura K, 1999 Grf40, A novel Grb2 family member, is involved in T cell signaling through interaction with SLP-76 and LAT. *J Exp Med* 189, 1383–1390. [PubMed: 10224278]
- Awasthi A, Samarakoon A, Chu H, Kamalakannan R, Quilliam LA, Chrzanowska-Wodnicka M, White GC 2nd, Malarkannan S, 2010 Rap1b facilitates NK cell functions via IQGAP1-mediated signalosomes. *J Exp Med* 207, 1923–1938. [PubMed: 20733035]
- Basu SK, Lee S, Salotti J, Basu S, Sakchaisri K, Xiao Z, Walia V, Westlake CJ, Morrison DK, Johnson PF, 2017 Oncogenic RAS-Induced Perinuclear Signaling Complexes Requiring KSR1 Regulate Signal Transmission to Downstream Targets. *Cancer Res.*

- Baud V, Liu ZG, Bennett B, Suzuki N, Xia Y, Karin M, 1999 Signaling by pro-inflammatory cytokines: oligomerization of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. *Genes Dev* 13, 1297–1308. [PubMed: 10346818]
- Bodmer JL, Schneider P, Tschopp J, 2002 The molecular architecture of the TNF superfamily. *Trends Biochem. Sci* 27, 19–26. [PubMed: 11796220]
- Boursalian TE, Fink PJ, 2003 Mutation in fas ligand impairs maturation of thymocytes bearing moderate affinity T cell receptors. *J Exp Med* 198, 349–360. [PubMed: 12860933]
- Bunnell SC, Diehn M, Yaffe MB, Findell PR, Cantley LC, Berg LJ, 2000 Biochemical interactions integrating Itk with the T cell receptor-initiated signaling cascade. *J Biol Chem* 275, 2219–2230. [PubMed: 10636929]
- Ching KA, Grasis JA, Tailor P, Kawakami Y, Kawakami T, Tsoukas CD, 2000 TCR/CD3-Induced activation and binding of Emt/Itk to linker of activated T cell complexes: requirement for the Src homology 2 domain. *J Immunol* 165, 256–262. [PubMed: 10861059]
- da Silva AJ, Li Z, de VC, Canto E, Findell P, Rudd CE, 1997 Cloning of a novel T-cell protein FYB that binds FYN and SH2-domain-containing leukocyte protein 76 and modulates interleukin 2 production. *Proc. Natl. Acad. Sci. U. S. A* 94, 7493–7498. [PubMed: 9207119]
- Dibble CC, Elis W, Menon S, Qin W, Klekota J, Asara JM, Finan PM, Kwiatkowski DJ, Murphy LO, Manning BD, 2012 TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of mTORC1. *Mol Cell* 47, 535–546. [PubMed: 22795129]
- Ding YW, Pan SY, Xie W, Shen HY, Wang HH, 2018 Elevated Soluble Fas and FasL in Cerebrospinal Fluid and Serum of Patients With Anti-N-methyl-D-aspartate Receptor Encephalitis. *Front Neurol* 9, 904. [PubMed: 30410466]
- Fruman DA, Snapper SB, Yballe CM, Davidson L, Yu JY, Alt FW, Cantley LC, 1999 Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85alpha. *Science* 283, 393–397. [PubMed: 9888855]
- Fukushima S, Matsuoka S, Ueda M, 2019 Excitable dynamics of Ras triggers spontaneous symmetry breaking of PIP3 signaling in motile cells. *J Cell Sci* 132.
- Gerbac ZJ, Thakar MS, Malarkannan S, 2015 The Fyn-ADAP Axis: Cytotoxicity Versus Cytokine Production in Killer Cells. *Front Immunol* 6, 472. [PubMed: 26441977]
- Geuking P, Narasimamurthy R, Lemaitre B, Basler K, Leulier F, 2009 A non-redundant role for *Drosophila* Mkk4 and hemipterous/Mkk7 in TAK1-mediated activation of JNK. *PLoS. ONE* 4, e7709. [PubMed: 19888449]
- Ghadimi MP, Sanzenbacher R, Thiede B, Wenzel J, Jing Q, Plomann M, Borkhardt A, Kabelitz D, Janssen O, 2002 Identification of interaction partners of the cytosolic polyproline region of CD95 ligand (CD178). *FEBS Lett* 519, 50–58. [PubMed: 12023017]
- Gorelik E, Edwards RP, Feng X, Marrangoni AM, Grandis JR, Drenning SD, Velikokhatnaya L, Kwon JA, Lokshin AE, 2004 IL-12 receptor-mediated upregulation of FasL in human ovarian carcinoma cells. *Int. J Cancer* 112, 620–627. [PubMed: 15382042]
- Grasis JA, Guimond DM, Cam NR, Herman K, Magotti P, Lambris JD, Tsoukas CD, 2010 In vivo significance of ITK-SLP-76 interaction in cytokine production. *Mol Cell Biol* 30, 3596–3609. [PubMed: 20457812]
- Greenblatt MB, Shim JH, Zou W, Sitara D, Schweitzer M, Hu D, Lotinun S, Sano Y, Baron R, Park JM, Arthur S, Xie M, Schneider MD, Zhai B, Gygi S, Davis R, Glimcher LH, 2010 The p38 MAPK pathway is essential for skeletogenesis and bone homeostasis in mice. *J Clin. Invest* 120, 2457–2473. [PubMed: 20551513]
- Gregory-Ksander M, Perez VL, Marshak-Rothstein A, Ksander BR, 2019 Soluble Fas ligand blocks destructive corneal inflammation in mouse models of corneal epithelial debridement and LPS induced keratitis. *Exp Eye Res* 179, 47–54. [PubMed: 30365944]
- Gregory MS, Hackett CG, Abernathy EF, Lee KS, Saff RR, Hohlbaum AM, Moody KS, Hobson MW, Jones A, Kolovou P, Karray S, Giani A, John SW, Chen DF, Marshak-Rothstein A, Ksander BR, 2011 Opposing roles for membrane bound and soluble Fas ligand in glaucoma-associated retinal ganglion cell death. *PLoS One* 6, e17659. [PubMed: 21479271]

- Gregory MS, Saff RR, Marshak-Rothstein A, Ksander BR, 2007 Control of ocular tumor growth and metastatic spread by soluble and membrane Fas ligand. *Cancer Res* 67, 11951–11958. [PubMed: 18089826]
- Hane M, Lowin B, Peitsch M, Becker K, Tschopp J, 1995 Interaction of peptides derived from the Fas ligand with the Fyn-SH3 domain. *FEBS Lett* 373, 265–268. [PubMed: 7589480]
- Herrero R, Kajikawa O, Matute-Bello G, Wang Y, Hagimoto N, Mongovin S, Wong V, Park DR, Brot N, Heinecke JW, Rosen H, Goodman RB, Fu X, Martin TR, 2011 The biological activity of FasL in human and mouse lungs is determined by the structure of its stalk region. *J Clin Invest* 121, 1174–1190. [PubMed: 21285513]
- Hohlbaum AM, Moe S, Marshak-Rothstein A, 2000 Opposing effects of transmembrane and soluble Fas ligand expression on inflammation and tumor cell survival. *J Exp Med* 191, 1209–1220. [PubMed: 10748238]
- Holler N, Tardivel A, Kovacovics-Bankowski M, Hertig S, Gaide O, Martinon F, Tinel A, Deperthes D, Calderara S, Schulthess T, Engel J, Schneider P, Tschopp J, 2003 Two adjacent trimeric Fas ligands are required for Fas signaling and formation of a death-inducing signaling complex. *Mol. Cell Biol* 23, 1428–1440. [PubMed: 12556501]
- Inoki K, Li Y, Zhu T, Wu J, Guan KL, 2002 TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 4, 648–657. [PubMed: 12172553]
- Inoki K, Zhu T, Guan KL, 2003 TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115, 577–590. [PubMed: 14651849]
- Ishimaru N, Yanagi K, Ogawa K, Suda T, Saito I, Hayashi Y, 2001 Possible role of organ-specific autoantigen for Fas ligand-mediated activation-induced cell death in murine Sjogren's syndrome. *J Immunol* 167, 6031–6037. [PubMed: 11698484]
- Kesti T, Ruppelt A, Wang JH, Liss M, Wagner R, Tasken K, Saksela K, 2007 Reciprocal regulation of SH3 and SH2 domain binding via tyrosine phosphorylation of a common site in CD3epsilon. *J Immunol* 179, 878–885. [PubMed: 17617578]
- Kreuz S, Siegmund D, Rumpf JJ, Samel D, Leverkus M, Janssen O, Hacker G, Trich-Breiholz O, Kracht M, Scheurich P, Wajant H, 2004 NF-kappaB activation by Fas is mediated through FADD, caspase-8, and RIP and is inhibited by FLIP. *J Cell Biol* 166, 369–380. [PubMed: 15289496]
- Krishnan A, Fei F, Jones A, Busto P, Marshak-Rothstein A, Ksander BR, Gregory-Ksander M, 2016 Overexpression of Soluble Fas Ligand following Adeno-Associated Virus Gene Therapy Prevents Retinal Ganglion Cell Death in Chronic and Acute Murine Models of Glaucoma. *J Immunol* 197, 4626–4638. [PubMed: 27849168]
- Law CL, Ewings MK, Chaudhary PM, Solow SA, Yun TJ, Marshall AJ, Hood L, Clark EA, 1999 GrpL, a Grb2-related adaptor protein, interacts with SLP-76 to regulate nuclear factor of activated T cell activation. *J Exp Med* 189, 1243–1253. [PubMed: 10209041]
- Lettau M, Qian J, Linkermann A, Latreille M, Larose L, Kabelitz D, Janssen O, 2006 The adaptor protein Nck interacts with Fas ligand: Guiding the death factor to the cytotoxic immunological synapse. *Proc. Natl. Acad. Sci. U. S. A* 103, 5911–5916. [PubMed: 16595635]
- Liu J, Kang H, Raab M, da Silva AJ, Kraeft SK, Rudd CE, 1998 FYB (FYN binding protein) serves as a binding partner for lymphoid protein and FYN kinase substrate SKAP55 and a SKAP55-related protein in T cells. *Proc. Natl. Acad. Sci. U. S. A* 95, 8779–8784. [PubMed: 9671755]
- Liu P, Gan W, Chin YR, Ogura K, Guo J, Zhang J, Wang B, Blenis J, Cantley LC, Toker A, Su B, Wei W, 2015 PtdIns(3,4,5)P3-Dependent Activation of the mTORC2 Kinase Complex. *Cancer Discov* 5, 1194–1209. [PubMed: 26293922]
- Liu W, Ramagopal U, Cheng H, Bonanno JB, Toro R, Bhosle R, Zhan C, Almo SC, 2016 Crystal Structure of the Complex of Human FasL and Its Decoy Receptor DcR3. *Structure* 24, 2016–2023. [PubMed: 27806260]
- Liu WH, Chang LS, 2011 Fas/FasL-dependent and -independent activation of caspase-8 in doxorubicin-treated human breast cancer MCF-7 cells: ADAM10 down-regulation activates Fas/FasL signaling pathway. *Int J Biochem Cell Biol* 43, 1708–1719. [PubMed: 21854868]
- Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J, 2005 Rheb binds and regulates the mTOR kinase. *Curr Biol* 15, 702–713. [PubMed: 15854902]

- Luckerath K, Kirkin V, Melzer IM, Thalheimer FB, Siele D, Milani W, Adler T, guilar-Pimentel A, Horsch M, Michel G, Beckers J, Busch DH, Ollert M, Gailus-Durner V, Fuchs H, Hrabe de AM, Staal FJ, Rajalingam K, Hueber AO, Strobl LJ, Zimmer-Strobl U, Zornig M, 2011 Immune modulation by Fas ligand reverse signaling: lymphocyte proliferation is attenuated by the intracellular Fas ligand domain. *Blood* 117, 519–529. [PubMed: 20971954]
- Ma L, Chen Z, Erdjument-Bromage H, Tempst P, Pandolfi PP, 2005 Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. *Cell* 121, 179–193. [PubMed: 15851026]
- Machida K, Thompson CM, Dierck K, Jablonowski K, Karkkainen S, Liu B, Zhang H, Nash PD, Newman DK, Nollau P, Pawson T, Renkema GH, Saksela K, Schiller MR, Shin DG, Mayer BJ, 2007 High-throughput phosphotyrosine profiling using SH2 domains. *Mol Cell* 26, 899–915. [PubMed: 17588523]
- Malarkannan S, Awasthi A, Rajasekaran K, Kumar P, Schuldt KM, Bartoszek A, Manoharan N, Goldner NK, Umhoefer CM, Thakar MS, 2012 IQGAP1: a regulator of intracellular spacetime relativity. *J Immunol* 188, 2057–2063. [PubMed: 22345702]
- Malarkannan S, Regunathan J, Chu H, Kutlesa S, Chen Y, Zeng H, Wen R, Wang D, 2007 Bcl10 plays a divergent role in NK cell-mediated cytotoxicity and cytokine generation. *J. Immunol* 179, 3752–3762. [PubMed: 17785812]
- Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC, 2002 Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberlin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 10, 151–162. [PubMed: 12150915]
- Matsuki Y, Li L, Hsu HC, Yang PA, Zheng R, Edwards CK 3rd, Chaudry IH, Zhang HG, Mountz JD, 2002 Soluble Fas gene therapy protects against Fas-mediated apoptosis of hepatocytes but not the lethal effects of Fas-induced TNF-alpha production by Kupffer cells. *Cell Death Differ* 9, 626–635. [PubMed: 12032671]
- Matsumoto H, Murakami Y, Kataoka K, Notomi S, Mantopoulos D, Trichonas G, Miller JW, Gregory MS, Ksander BR, Marshak-Rothstein A, Vavvas DG, 2015 Membrane-bound and soluble Fas ligands have opposite functions in photoreceptor cell death following separation from the retinal pigment epithelium. *Cell Death Dis* 6, e1986. [PubMed: 26583327]
- Medeiros RB, Burbach BJ, Mueller KL, Srivastava R, Moon JJ, Highfill S, Peterson EJ, Shimizu Y, 2007 Regulation of NF-kappaB activation in T cells via association of the adapter proteins ADAP and CARMA1. *Science* 316, 754–758. [PubMed: 17478723]
- Miller AT, Berg LJ, 2002 Defective Fas ligand expression and activation-induced cell death in the absence of IL-2-inducible T cell kinase. *J Immunol* 168, 2163–2172. [PubMed: 11859102]
- Montel AH, Bochan MR, Hobbs JA, Lynch DH, Brahmi Z, 1995 Fas involvement in cytotoxicity mediated by human NK cells. *Cell Immunol* 166, 236–246. [PubMed: 7497525]
- Morlon A, Munnich A, Smahi A, 2005 TAB2, TRAF6 and TAK1 are involved in NF-kappaB activation induced by the TNF-receptor, Edar and its adaptator Edaradd. *Hum. Mol Genet* 14, 3751–3757. [PubMed: 16251197]
- Nagata S, Golstein P, 1995 The Fas death factor. *Science* 267, 1449–1456. [PubMed: 7533326]
- Nagata S, Suda T, 1995 Fas and Fas ligand: lpr and gld mutations. *Immunol. Today* 16, 39–43. [PubMed: 7533498]
- Ninomiya-Tsuji J, Kishimoto K, Hiyama A, Inoue J, Cao Z, Matsumoto K, 1999 The kinase TAK1 can activate the NIK-I kappaB as well as the MAP kinase cascade in the IL-1 signalling pathway. *Nature* 398, 252–256. [PubMed: 10094049]
- Peterson EJ, Woods ML, Dmowski SA, Derimanov G, Jordan MS, Wu JN, Myung PS, Liu QH, Pribila JT, Freedman BD, Shimizu Y, Koretzky GA, 2001 Coupling of the TCR to integrin activation by Slap-130/Fyb. *Science* 293, 2263–2265. [PubMed: 11567141]
- Rajasekaran K, Chu H, Kumar P, Xiao Y, Tinguely M, Samarakoon A, Kim TW, Li X, Thakar MS, Zhang J, Malarkannan S, 2011a Transforming Growth Factor- β -activated Kinase 1 Regulates Natural Killer Cell-mediated Cytotoxicity and Cytokine Production. *J Biol. Chem* 286, 31213–31224. [PubMed: 21771792]
- Rajasekaran K, Chu H, Kumar P, Xiao Y, Tinguely M, Samarakoon A, Kim TW, Li X, Thakar MS, Zhang J, Malarkannan S, 2011b Transforming growth factor-beta-activated kinase 1 regulates

natural killer cell-mediated cytotoxicity and cytokine production. *J Biol Chem* 286, 31213–31224. [PubMed: 21771792]

- Rajasekaran K, Kumar P, Schuldt KM, Peterson EJ, Vanhaesebroeck B, Dixit V, Thakar MS, Malarkannan S, 2013 Signaling by Fyn-ADAP via the Carma1-Bcl-10-MAP3K7 signalosome exclusively regulates inflammatory cytokine production in NK cells. *Nat. Immunol* 14, 1127–1136. [PubMed: 24036998]
- Rajasekaran K, Riese MJ, Rao S, Wang L, Thakar MS, Sentman CL, Malarkannan S, 2016 Signaling in Effector Lymphocytes: Insights toward Safer Immunotherapy. *Front Immunol* 7, 176. [PubMed: 27242783]
- Refaeli Y, Van Parijs L, London CA, Tschopp J, Abbas AK, 1998 Biochemical mechanisms of IL-2-regulated Fas-mediated T cell apoptosis. *Immunity* 8, 615–623. [PubMed: 9620682]
- Roth W, Isenmann S, Nakamura M, Platten M, Wick W, Kleihues P, Bahr M, Ohgaki H, Ashkenazi A, Weller M, 2001 Soluble decoy receptor 3 is expressed by malignant gliomas and suppresses CD95 ligand-induced apoptosis and chemotaxis. *Cancer Res* 61, 2759–2765. [PubMed: 11289159]
- Roths JB, Murphy ED, Eicher EM, 1984 A new mutation, gld, that produces lymphoproliferation and autoimmunity in C3H/HeJ mice. *J Exp. Med* 159, 1–20. [PubMed: 6693832]
- Roux PP, Ballif BA, Anjum R, Gygi SP, Blenis J, 2004 Tumor-promoting phorbol esters and activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6 kinase. *Proc Natl Acad Sci U S A* 101, 13489–13494. [PubMed: 15342917]
- Sarbassov DD, Guertin DA, Ali SM, Sabatini DM, 2005 Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307, 1098–1101. [PubMed: 15718470]
- Saxena A, Yagita H, Donner TW, Hamad ARA, 2017 Expansion of FasL-Expressing CD5(+) B Cells in Type 1 Diabetes Patients. *Front Immunol* 8, 402. [PubMed: 28439273]
- Schulze-Luehrmann J, Ghosh S, 2006 Antigen-receptor signaling to nuclear factor kappa B. *Immunity* 25, 701–715. [PubMed: 17098202]
- Shao RG, Cao CX, Nieves-Neira W, Dimanche-Boitrel MT, Solary E, Pommier Y, 2001 Activation of the Fas pathway independently of Fas ligand during apoptosis induced by camptothecin in p53 mutant human colon carcinoma cells. *Oncogene* 20, 1852–1859. [PubMed: 11313933]
- Shrestha B, Diamond MS, 2007 Fas ligand interactions contribute to CD8+ T-cell-mediated control of West Nile virus infection in the central nervous system. *J Virol* 81, 11749–11757. [PubMed: 17804505]
- Srivastava R, Burbach BJ, Shimizu Y, 2010 NF-kappaB activation in T cells requires discrete control of IkappaB kinase alpha/beta (IKKalpha/beta) phosphorylation and IKKgamma ubiquitination by the ADAP adapter protein. *J Biol. Chem* 285, 11100–11105. [PubMed: 20164171]
- Suda T, Takahashi T, Golstein P, Nagata S, 1993 Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 75, 1169–1178. [PubMed: 7505205]
- Sun M, Ames KT, Suzuki I, Fink PJ, 2006 The cytoplasmic domain of Fas ligand costimulates TCR signals. *J Immunol* 177, 1481–1491. [PubMed: 16849454]
- Sun M, Lee S, Karray S, Levi-Strauss M, Ames KT, Fink PJ, 2007 Cutting edge: two distinct motifs within the Fas ligand tail regulate Fas ligand-mediated costimulation. *J Immunol* 179, 5639–5643. [PubMed: 17947633]
- Suzuki H, Terauchi Y, Fujiwara M, Aizawa S, Yazaki Y, Kadowaki T, Koyasu S, 1999 Xid-like immunodeficiency in mice with disruption of the p85alpha subunit of phosphoinositide 3-kinase. *Science* 283, 390–392. [PubMed: 9888854]
- Suzuki I, Fink PJ, 1998 Maximal proliferation of cytotoxic T lymphocytes requires reverse signaling through Fas ligand. *J Exp. Med* 187, 123–128. [PubMed: 9419218]
- Suzuki I, Fink PJ, 2000 The dual functions of fas ligand in the regulation of peripheral CD8+ and CD4+ T cells. *Proc. Natl. Acad. Sci. U. S. A* 97, 1707–1712. [PubMed: 10677522]
- Suzuki I, Martin S, Boursalian TE, Beers C, Fink PJ, 2000 Fas ligand costimulates the in vivo proliferation of CD8+ T cells. *J Immunol* 165, 5537–5543. [PubMed: 11067907]
- Takahama Y, Yamada Y, Emoto K, Fujimoto H, Takayama T, Ueno M, Uchida H, Hirao S, Mizuno T, Nakajima Y, 2002 The prognostic significance of overexpression of the decoy receptor for Fas ligand (DcR3) in patients with gastric carcinomas. *Gastric Cancer* 5, 61–68. [PubMed: 12111580]

- Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J, 2003 Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol* 13, 1259–1268. [PubMed: 12906785]
- Thakar MS, Kearn TJ, Malarkannan S, 2019 Controlling Cytokine Release Syndrome to Harness the Full Potential of CAR-Based Cellular Therapy. *Front Oncol* 9, 1529. [PubMed: 32076597]
- Thornhill PB, Cohn JB, Drury G, Stanford WL, Bernstein A, Desbarats J, 2007 A proteomic screen reveals novel Fas ligand interacting proteins within nervous system Schwann cells. *FEBS Lett* 581, 4455–4462. [PubMed: 17761170]
- Thornhill PB, Cohn JB, Stanford WL, Desbarats J, 2008 The adaptor protein Grb2 regulates cell surface Fas ligand in Schwann cells. *Biochem. Biophys. Res. Commun* 376, 341–346. [PubMed: 18789888]
- Upshaw JL, Arneson LN, Schoon RA, Dick CJ, Billadeau DD, Leibson PJ, 2006 NKG2D-mediated signaling requires a DAP10-bound Grb2-Vav1 intermediate and phosphatidylinositol-3-kinase in human natural killer cells. *Nat Immunol* 7, 524–532. [PubMed: 16582911]
- Vanhaesebroeck B, Ali K, Bilancio A, Geering B, Foukas LC, 2005 Signalling by PI3K isoforms: insights from gene-targeted mice. *Trends Biochem. Sci* 30, 194–204. [PubMed: 15817396]
- Vanhaesebroeck B, Guillemet-Guibert J, Graupera M, Bilanges B, 2010 The emerging mechanisms of isoform-specific PI3K signalling. *Nat. Rev. Mol. Cell Biol* 11, 329–341. [PubMed: 20379207]
- Vanhaesebroeck B, Leever SJ, Ahmadi K, Timms J, Katso R, Driscoll PC, Woscholski R, Parker PJ, Waterfield MD, 2001 Synthesis and function of 3-phosphorylated inositol lipids. *Annu. Rev. Biochem* 70, 535–602. [PubMed: 11395417]
- Vanhaesebroeck B, Rohn JL, Waterfield MD, 2004 Gene targeting: attention to detail. *Cell* 118, 274–276. [PubMed: 15294152]
- Ventura JJ, Kennedy NJ, Lamb JA, Flavell RA, Davis RJ, 2003 c-Jun NH(2)-terminal kinase is essential for the regulation of AP-1 by tumor necrosis factor. *Mol. Cell Biol* 23, 2871–2882. [PubMed: 12665585]
- Voss M, Lettau M, Janssen O, 2009 Identification of SH3 domain interaction partners of human FasL (CD178) by phage display screening. *BMC. Immunol* 10, 53. [PubMed: 19807924]
- Wallin RP, Screpanti V, Michaelsson J, Grandien A, Ljunggren HG, 2003 Regulation of perforin-independent NK cell-mediated cytotoxicity. *Eur J Immunol* 33, 2727–2735. [PubMed: 14515256]
- Watts TH, DeBenedette MA, 1999 T cell co-stimulatory molecules other than CD28. *Curr. Opin. Immunol* 11, 286–293. [PubMed: 10375549]
- Wenzel J, Sanzenbacher R, Ghadimi M, Lewitzky M, Zhou Q, Kaplan DR, Kabelitz D, Feller SM, Janssen O, 2001 Multiple interactions of the cytosolic polyproline region of the CD95 ligand: hints for the reverse signal transduction capacity of a death factor. *FEBS Lett* 509, 255–262. [PubMed: 11741599]
- Yablonski D, Kadlec T, Weiss A, 2001 Identification of a phospholipase C-gamma1 (PLC-gamma1) SH3 domain-binding site in SLP-76 required for T-cell receptor-mediated activation of PLC-gamma1 and NFAT. *Mol Cell Biol* 21, 4208–4218. [PubMed: 11390650]
- Yamada A, Arakaki R, Saito M, Kudo Y, Ishimaru N, 2017 Dual Role of Fas/FasL-Mediated Signal in Peripheral Immune Tolerance. *Front Immunol* 8, 403. [PubMed: 28424702]
- Yang G, Murashige DS, Humphrey SJ, James DE, 2015 A Positive Feedback Loop between Akt and mTORC2 via SIN1 Phosphorylation. *Cell Rep* 12, 937–943. [PubMed: 26235620]
- Zhu Y, Huang B, Shi J, 2016 Fas ligand and lytic granule differentially control cytotoxic dynamics of natural killer cell against cancer target. *Oncotarget* 7, 47163–47172. [PubMed: 27323411]

Highlights

Fas-FasL is one of the major molecular interactions between infected/transformed and the immune cells. Effector lymphocytes, including NK and T cells, express FasL. Expression of Fas, the receptor for FasL in tumor cells, renders them susceptible to NK and T cell-mediated killing. The functional relevance of FasL in initiating death signals in tumor cells is well-characterized. However, the cytoplasmic interacting partners and the potential signaling pathways downstream of FasL are far from fully defined. FasL possesses an 81 amino acid long cytoplasmic tail with multiple unique recruitment motifs. We predict two major, interdependent signaling complexes that form the core of the 'reverse signaling' downstream of FasL.

The FasL possesses binding sites for Class I Src homology 3 (SH3)-containing proteins, including Fyn. Fyn recruits and phosphorylates the regulatory p85 α subunit of PI(3)K, which associates with the catalytic p110 δ subunit of PI(3)K. Downstream of PI(3)K, the MAPK, mTORC1, and mTORC2 can be activated. Thus, FasL can activate signaling cascades responsible for a variety of cellular functions, including cell development, cell-mediated cytotoxicity, and inflammatory cytokine/chemokine generation.

Figure 3. FasL initiates a Fyn-ADAP-CBM signalosome to regulate inflammatory cytokine production.

Following the recruitment and activation of Fyn by FasL, the SH2 domain of Fyn can interact with the pYDGI motif of ADAP with a high affinity. This translocates ADAP to the plasma membrane. Membrane-bound ADAP directly recruits Carma1 and Tak1. This results in the formation of the Carma1-Bcl10-Malt1-based CBM signalosome that is essential for the activation and nuclear translocation of NF- κ B and AP-1. These transcription factors are the major regulators of multiple cellular functions, including the generation of key inflammatory cytokines and chemokines.