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Clnk plays a role in TNF-alpha-induced cell death in murine fibrosarcoma cell line L929

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ABSTRACT

Clnk, as a third member of the Blnk/SLP-76 adapter family, is involved in the positive regulation of immunoreceptor signaling. Here we provide findings that Clnk may be is required for TNF induced cell death, it functions downstream of RIP3 and promotes TNF- induced ROS generation and MLKL tetramer formation and subsequent necrosis of L929 cells. Therefore, Clnk, as an adaptor protein, may take part in the other cellular processes.

1. Introduction

Murine Clnk/MIST(for cytokine-dependent hemopoietic cell linker/mast cell immunoreceptor signal transducer), a 435 amino acid protein, is a third member of the Blnk/SLP-76 adapter family [1]. Early study reports that Clnk is expressed in a variety of cytokine-dependent hematopoietic cell lines of myeloid and lymphoid origin as well as some cytokine-independent mast cell lines [[2,3] and its transcription may be regulated by STAT5A in cytokine-dependent fashion [4]. In common with other family members, Clnk is composed of an N-terminal acidic domain , two central proline-rich regions(PR1 and PR2), and C-terminal SH2 domain. The two amino-terminus tyrosine residues(Y69 and Y96) can be phosphorylated by Src family PTKs Lyn or Syk and PR1 is required to interact with an SH3 domain of PLCγ while PR2 is involved in the association with Grb2 [5]. The C-terminal SH2 domain of Clnk acts as a binding site for ADAP (previously known as SLAP-130/Fyb) [6] as well as a hematopoietic serine/threonine kinase, HPK-1 [7].

Similar to its relatives, Clnk is involved in the positive regulation of immunoreceptor signaling. Overexpression of Clnk in SLP-76-deficient J14 cells can rescue antigen receptor-induced activation of report genes [7]. Similarly, coexpression of Clnk with LAT in BLNK-deficient DT40 chicken B cells almost fully restores their B cell antigen receptor (BCR) signaling [5].

As described by Utting et al., Clnk deficient mice [8] are viable and healthy, and not essential for normal differentiation and function of T cell, NK cell, and mast cell. Interesting, IFN-γ production is enhanced in Clnk deficient NK cells. In contrast to NK cell, Clnk deficient $CD4^+$ NKT cells show a reduced IFN-γ production. Further studies demonstrate that the Src family kinase, Fgr, is differentially expressed in the two cell types and the interaction of Clnk with Fgr suppresses NK-cell receptor–induced IFN-γ production [9].

Here we have extended our knowledge about the function of the Clnk protein beyond its primary role in response to immunoreceptor signaling. Specifically we provide findings that Clnk may be is required for TNF-α-induced cell death, it functions downstream of RIP3 and promotes TNF- induced ROS generation and MLKL tetramer formation and subsequent necrosis of L929 cells.

2. Materials and methods

2.1. Reagens

Mouse TNF-α and IL-2 were obtained from Sigma. Dihydroethidium(HE) and Dichlorofluorescin-diacetate(DCFH-DA) are from Molecular Probes. The following antibodies were purchased: anti-FLAG monoclonal antibody affinity gel M2 from Sigma, anti-flag monoclonal antibody, anti-c-Myc pAb, anti-HA pAb and

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Cy3-goat anti-mouse IgG from ABclonal Biotechnology, horseradish peroxidase-labeled goat anti-mouse or anti-rabbit IgG secondary Ab from Pierce.

2.2. Cell culture and transfection

All cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (Hyclone) and 100 ug/ml penicillin and streptomycin at 37 °C in a humidified incubator with 5% CO_2. 293T cells were transiently transfected with 2 μg (total) of plasmids using Lipofectamine 2000 transfection reagent(Invitrogen).

2.3. Immunoprecipitation and western blotting

Cells were lysed in lysis buffer with 1% Triton X-100, protease and phosphatase inhibitors, and incubated with anti-flag beads and gently shaken for 4 h at 4 °C. The beads were washed three times with the lysis buffer. The precipitates and total cell lysates were resolved on SDS-PAGE with or without β-mercaptoethanol(BME), transferred to PVDF membranes, and then immunoblotted with Abs described above and the secondary Ab conjugated with horseradish peroxidase. The presence of a protein band was visualized using enhanced chemiluminescence (ECL) detection reagents.

2.4. Identification of mutated gene and measurement of mRNA levels

For 3′ Race, total RNA was isolated using Tri-Zol reagent (Invitrogen) and converted into cDNA with oligo-(dT) primer (5′-CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGC $(T)_{17}$-3′). A nested PCR was performed with the resultant cDNA by using primers P1/Q1 (5′- AAAGCCATAGTGAAGGACAGT GA-3′/5′-CCAGTGAGCAGAGTG ACG-3′) and P2/Q2 (5′-TGCTGCCCTCTGGTTATGTGTGG-3′/5′-GAG GACT CGAGCTCAAGC-3′), respectively, as described previously [10]. P1 and P2 are located on the blasticidin S resistance gene, whereas Q1 and Q2 are on the anchor sequence of oligo-(dT) primer. The PCR fragments were subcloned into the pBKS(+) vector. Their sequences were verified by automated sequence analysis.

For clnk mRNA transcription, semiquantitative PCR was performed as outlined previously [10]. Primers for mouse clnk were as follows: 5′-GTTTCCTCGA GAGCAATCAA-3′/5′-CGAGGACTCGAG CTCAAGCTT-3′ or 5′-TCTTCTTGC CCATCATCAAA-3′/5′-CGAGGACTCGAGCTCAAGCTT-3′. Primers for human clnk were as follows: 5′- AAGTCCAGAGAATGCCCAGT-3′ and 5′-CATCTCCTCT GAGTCC TGT CC-3′.

2.5. Measurement of cell death

Cell survival rates were determined by flow cytometry as described previously [11]. Briefly, cells were trypsinized, collected by centrifugation, washed once with phosphate-buffered saline (PBS), and resuspended in PBS containing 1 mg/ml propidium iodide (PI, Sigma). The cell death was determined by their plasma membrane integrity which was evaluated by the ability of cells to exclude PI on a FACScan flow cytometer (Beckman coulter® EPICS XL™). Cell size was assessed by forward-angle light scattering. PI negative cells with a normal size were considered living.

2.6. Measurement of ROS

Generation of intracellular superoxides were determined by flow cytometry using the probes HE or DCFH-DA. HE is ready to be oxidized to the fluorescent ethidium by O_2^- while DCFH-DA in the cell is cleaved by nonspecific esterase and releases 2′-7′-dichlorodihydrofluorescein (DCFH2) in which in turn is oxidized by H_2O_2 to fluorescent 2′-7′-dichlorofluorescein (DCF) in the presence of a catalyst such as peroxidase, cytochrome *c*,Fe^{3+}. Thus, the fluorescence intensity of ethidium or DCF indicates O_2^- or H_2O_2 level and peroxides activity. Cells were trypsinized, collected and washed once with PBS. The pellets were resuspended in PBS containing 6.6 μM HE or 10 μM DCFH-DA and 1 μg/ml PI, incubated for 45 min at 37 °C. Fluorescence was measure by flow cytometry on a FACScan flow cytometer.

2.7. Lentivirus package

293T cells were used for virus production. Cells were plated in 6-cm dishes. The calcium phosphate precipitation method was used to transfect cells at 80% confluent with lentivirus vectors. Cells were changed to fresh medium 12 h later. The supernatant with viruses was harvested 36 h later for infection.

2.8. Immunofluorescence

HeLa Cells cultured on coverslips were fixed in PBS with 4% paraformaldehyde for 10 min at room temperature, permeabilized in PBS containing 0.2% Triton-X100 for 5 min, then blocked with 5% BSA for 1 h. Cells were incubated with anti-flag antibodies for 1 h, rinsed, and then incubated with Cy3-conjugated goat anti-mouse IgG. The samples were mounted and examined through fluorescent microscope.

3. Result and discussion

3.1. Clnk modulates necrosis in L929 cells

L929 cell line is frequently used as a model to study TNF-α induced cell death. We initially used it to identify genes required for TNF-α-induced cell death by using a retroviral-induced mutagenesis approach coupled with TNF treatment to select TNF-resistant cell lines generated by the viral insertion [11]. Retroviral integration can generate mutant alleles resulting in diminished or abolished expression of the target gene. Interestingly, the gene disrupted in one of the TNF-resistant cell lines was identified as *clnk*, we termed this clone $Clnk^{mut}$. The insertion was mapped in the *clnk* gene between exon 14 and 15. A partial sequence of the fused gene product generated by retroviral insertion in this line was shown in Fig. 1A. Semiquantitative PCR analysis revealed a reduced expression of clnk mRNA (Fig. 1B), confirming that one allele of the *clnk* gene had been disrupted. Fig. 1C showed that $Clnk^{mut}$ cells were resistant to TNF-induced cell death unlike parental wild-type L929 cells. To establish whether that the reduction of Clnk is responsible for TNF-resistance, we needed to reconstitute Clnk expression in $Clnk^{mut}$ cells. Lentiviruses can deliver genes into L929 cells with almost 100% efficiency (data not shown), and therefore were used to express Clnk in $Clnk^{mut}$ cells. Expression of Clnk in $Clnk^{mut}$ cells rescued TNF induced cell death, whereas empty vector transfection of $Clnk^{mut}$ cells had no effect on TNF sensitivity (Fig. 1D). The results presented demonstrated that Clnk was involved in TNF-induced cell death in L929 cells.

Cao et al. reported that cytokine can enhance clnk gene transcription in HT-2 cells [2], an IL-2-dependent T cell lines. We asked whether cytokine stimulation also induced the accumulation of clnk in nonhematopoietic L929 cells. To test this possibility, L929 cells were stimulated with IL-2 and TNF-α, respectively, the impact of cytokine stimulation on clnk expression was evaluated using semiquantitative RT PCR. The results showed that IL-2 and TNF-α didn't significantly enhance clnk expression[Fig. S1A and B]. The results indicated that TNF-induced cell death was not by increasing expression of Clnk in L929 cells.

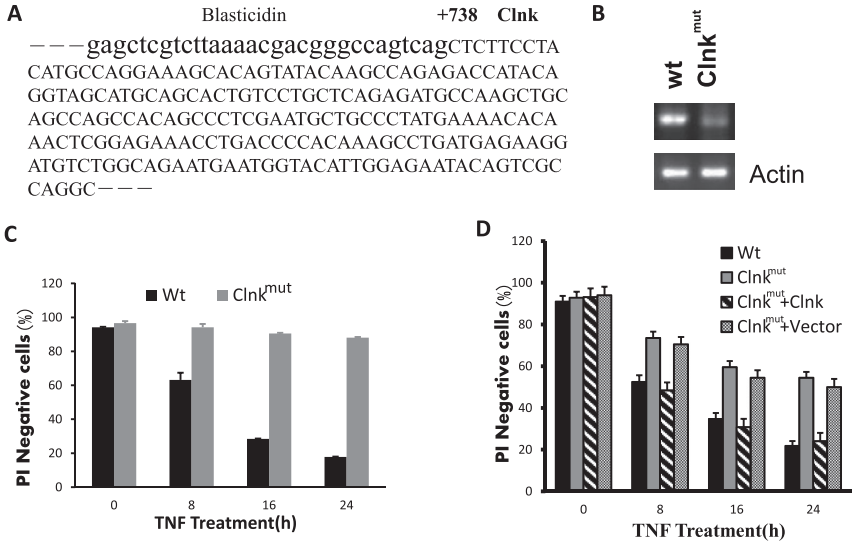

Fig. 1. Clnk mutation in L929 cells leads to a resistance of TNF-induced cell death. (**A**) The junction sequence of the fused cDNA of Blasticidin and an endogenous gene in a TNF-resistant L929 clonal cell line was shown. The viral insertion occurred at the coding region of the *clnk* gene. The sequence introduced by viral vectors was shown in lowercase. The number in parentheses indicated the position relative to the start codon of *clnk*. (**B**) *clnk* mRNA is reduced in Clnk mutant cells ($Clnk^{mut}$). A semiquantitative RT-PCR was performed using the total RNA from wild-type parental (Wt) and $Clnk^{mut}$ cells, Actin was used as control. (**C**) Wild-type and $Clnk^{mut}$ cells were treated with TNF (10 ng/ml) for different periods of time and cell viability was assessed by propidium iodide (PI) exclusion. Results represent the means ± s.e. (n = 3). (**D**) Stable cell lines were generated from $clnk^{mut}$ cells by infection with a lentivirus encoding Clnk, or empty vector and the resulting cells were designated as $Clnk^{mut}$ + Clnk and $Clnk^{mut}$ + Vector. Wild-type and $Clnk^{mut}$, $Clnk^{mut}$ + Clnk and $Clnk^{mut}$ + Vector cells were treated with TNF (10 ng/ml) for different periods of time and cell viability was assessed as in (**C**).

3.2. *Clnk is essential for TNF-induced ROS generation in L929 cells*

It has been shown that ROS production plays a crucial role in TNF-induced cell necrosis in L929 cells [11]. We measured oxidative burst and peroxidase activity with HE and DCFH-DA. As shown in Fig. 2, conversion of HE to ethidium or DCFH-DA to DCF increased in a time-dependent manner in TNF-treated wild-type cells, in contrast, the induction of ROS was significantly inhibited in $Clnk^{mut}$ cells. Since ROS generation is required for TNF-induced L929 cell necrosis, $Clnk^{mut}$ cell resistance to TNF- induced cell death most likely resulted from a blocking of TNF-induced ROS production.

Cell death is determined by a balance between survival and death pathways. NF-κB is known to promote cell survival in many cell systems. TNF activates several intracellular signaling pathways including the NF-κB and p38 MAP kinase pathways [11]. We examined whether TNF-induced NF-κB activation and p38 MAP kinase phosphorylation were affected by *clnk* allelic inactivation. As shown in Fig. S2, TNF-induced IκB degradation was comparable in $Clnk^{mut}$ and wild-type L929 cells, and TNF-induced p38 phosphorylation was also similar in wild-type and mutant cells. Therefore, Clnk deficiency does not affect survival pathway, but impairs TNF-activated death signal transduction.

3.3. *Clnk is required for cell death signal transduction downstream of RIP3*

TNF-induced L929 cell death is initiated by TNFR1. The ligation of TNFR1 leads to the assembly of the complex named as necrosome containing RIP1 and RIP3(receptor interacting protein 1 and 3), which delivers a pronecrosis signal [12]. It is also known that RIP3 overexpression of can kill cells [13]. We examined whether Clnk deficiency had any effect on cell death mediated by overexpression of RIP3. RIP3 was transiently expressed in wild-type and $Clnk^{mut}$ L929 cells by infection with a lentivirus encoding Clnk and cell viability was determined. RIP3 overexpression did lead to significant cell death in the wild-type but not in the mutant cells (Fig. 3). Since $Clnk^{mut}$ cells were resistant to RIP3 overexpression

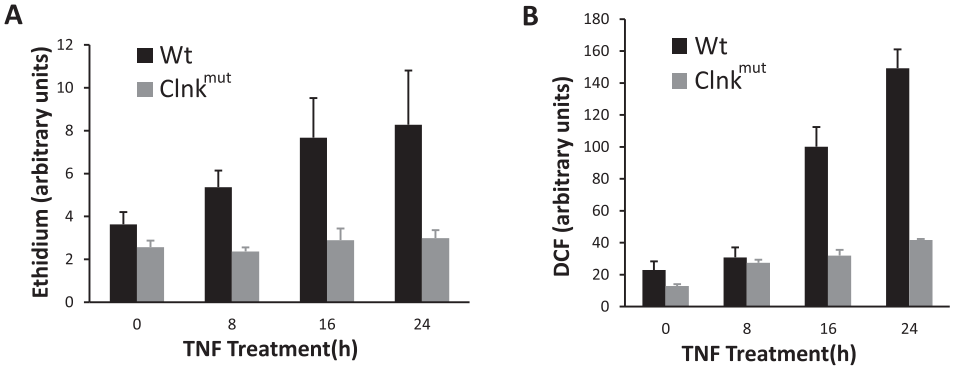

Fig. 2. Clnk deficiency blocks TNF-induced ROS generation. Wild-type and $Clnk^{mut}$ cells were stained with hydroethidine (HE) or dichloro-fluoresceindiacetate (DCFH-DA) together with PI at different times of TNF treatment(10 ng/ml). Levels of ethidium, the oxidation product of HE, or dichlorofluorescein (DCF), the fluorescent product of DCFH-DA, in PI-negative cells (live cells) were determined and shown. Results represent the means ± s.e. (n = 3).

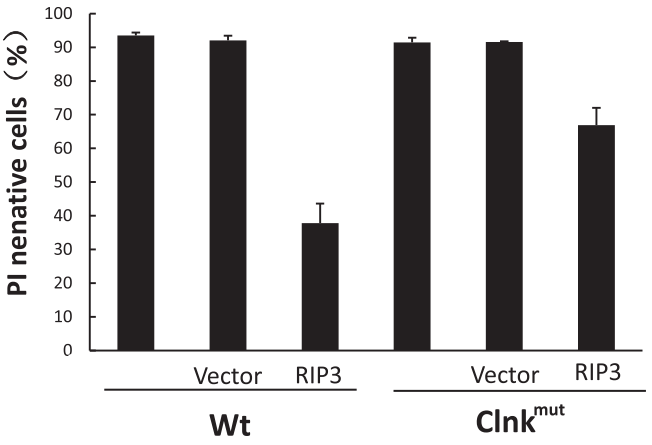

Fig. 3. Clnk deficiency interferes with cell death signal transduction downstream of RIP3. RIP3 was transiently expressed in wild-type and Clnk^{mut} L929 cells by infection with a lentivirus encoding RIP3. Cell viability was measured by using flow cytometry based on propidium iodide (PI) staining at 8 h after infection. Results represent the means ± s.e(n = 3).

induced cell death and Clnk did not interact with RIP3(data not shown), Clnk may relay cell death signaling downstream of RIP3.

3.4. Clnk promotes MLKL oligomerization

A recent study suggested that mixed lineage kinase domain-like protein (MLKL) functions downstream of RIP3 in TNF-α -induced necrosis [14,15]. The oligomerization of MLKL in TNF-treated cells is essential for necrosis. The translocation of MLKL tetramers to the plasma membrane leads to membrane rupture and sequential cell death [16–18]. Therefore, we analyzed whether Clnk deficiency has also the effect on MLKL oligomerization. Lentiviruses were used to express C-terminal flag-tagged MLKL in wild and Clnk^{mut} L929 cells. Overexpression of MLKL did not induce cell death in both cell lines (Fig. S3). After the treatment with TNF + zVAD for 3 h, MLKL tetramers can form in wild cells but not in Clnk^{mut} cells (Fig. 4A). We observed also the translocation of MLKL to the plasma membrane through immune-fluorescence microscope. MLKL can translocate to the plasma membrane in wild and Clnk^{mut} cells. But, since Clnk^{mut} cells were resistant to TNF stimulation(Fig. S3), less cells were visualized with MLKL translocation to the plasma membrane

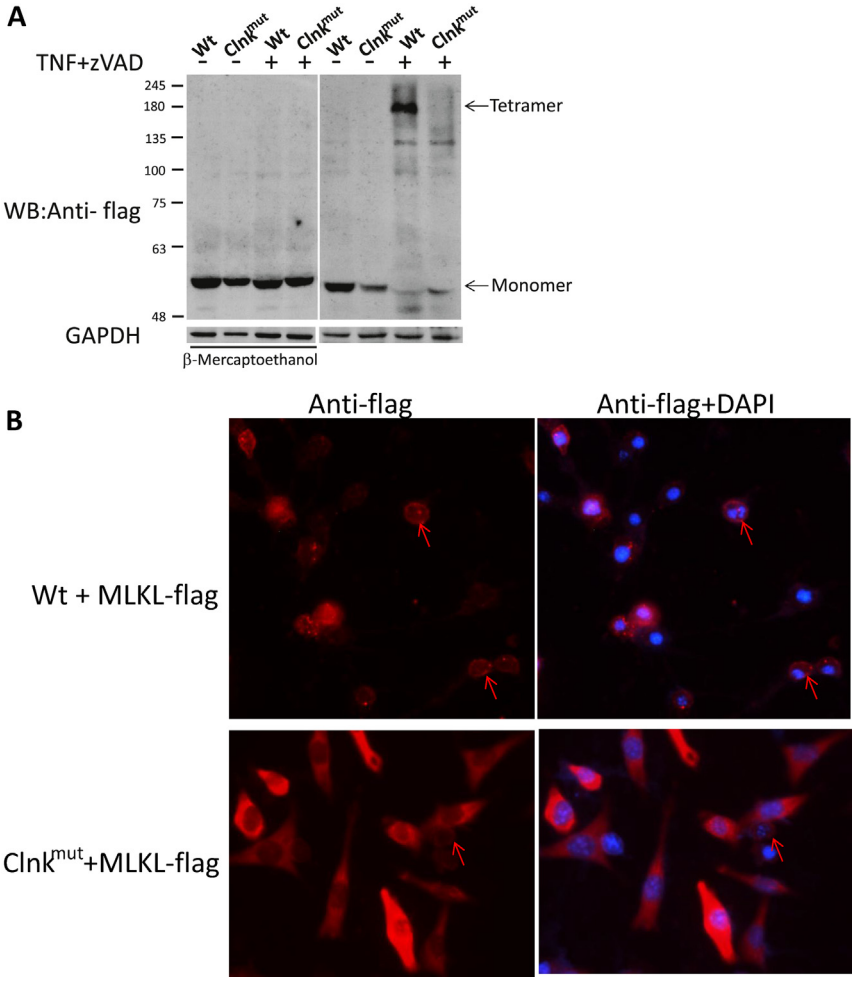

Fig. 4. Clnk deficiency decreases MLKL oligomerization. (A) Wild-type and Clnk^{mut} L929 cells expressing MLKL-flag were stimulated with or without TNF + zVAD (10 ng/ml + 20 μM) for 3 h, lysed and subjected to SDS-PAGE with or without β-mercaptoethanol (BME), and analyzed by immunoblotting using the anti-flag antibody. Arrows indicate the monomer or tetramer of MLKL-flag. (B) Wild-type and Clnk^{mut} L929 cells expressing MLKL-flag were stimulated with or without TNF + zVAD (10 ng/ml + 20 μM) for 3.5 h, and then immunostained for flag and counterstained with DAPI. Arrows indicated MLKL-flag oligomer located in the plasma membrane.

compared to the wild cells (Fig. 4B). MLKL phosphorylation at S345 is critical for its oligomerization [15,18], it is possible that Clnk deficiency diminished MLKL phosphorylation at that site.

Early study showed that expression of clnk was restricted to cytokine-dependent hemopoietic cell lines such as IL-2–dependent T cell line HT-2, and the IL-3–dependent pro-B cell line Ba/F3IL-3, and to cytokine-independent cell lines, the mastocytoma cell line P815, and the primitive leukemia cell line L1210 [2]. By RT-PCR analysis, we found that murine clnk, except expressed in L929 cells, was detectable in mouse skin melanoma cell line B16–F10 [Fig. S4A] and that human clnk was expressed in a variety of human normal and cancer cell lines, including human hepatocellular liver carcinoma cell line Hep G2, human colon cancer cell line HCT116, human lung adenocarcinoma epithelial cell line A549, human non-small cell lung carcinoma cell line H1299, human breast adenocarcinoma cell line MCF7, human cervical cancer cell line Hela, human promyelocytic leukemia cell line HL-60, and even in human retinal pigment epithelial cell line ARPE-19 and 293T[Fig. S4B]. Thus, Clnk can be expressed in a variety of cytokine-independent nonhematopoietic cell lines constitutely and may be related to the other cellular biological processes. Therefore, it is not surprising that the Clnk was involved in the cell death processes in L929 cells.

Since Clnk did not interact with RIP3 and MLKL(data not shown). We attempted to identify the proteins which may be involved in Clnk-mediated cell death. With liquid chromatography–tandem mass spectrometry (LC-MS/MS) and immunoprecipitation, we found that Several proteins such as Lamin B receptor(LBR) and Argonaute 2(Ago2), central protein component of RNA-induced silencing complex (RISC) [20], were in the Clnk complex. Further, human Ago2 and LBR were confirmed to interact with human Clnk, respectively, by means of coimmunoprecipitation of coexpressed proteins in 293T cells [Fig. S5A and B]. Moreover, the N-terminal fragment(1-208aa) of LBR can interact with DNA and protein such as lamin B and HP1 [19], but not with Clnk [Fig. S5A]. Human Clnk was distributed in the cytoplasm and colocolized with LBR at nuclear envelope in HeLa cells [Fig. S5C]. But, whether and how LBR and Ago2 were related to Clnk-mediated cell death remained unknown.

In summary, our findings suggested that clnk, except mediating immunoreceptor signaling as an adaptor protein, may be is required for TNF induced cell death, it functions downstream of RIP3 and promotes TNF- induced ROS generation and MLKL tetramer formation and subsequent necrosis of L929 cells. Clnk is a multi-function protein.

Conflict of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.05.046.

Transparency document

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References

[1] J.N. Wu, G.A. Koretzky, The SLP-76 family of adapter proteins, Semin. Immunol. 16 (2004) 379–393.
[2] M.Y. Cao, D. Davidson, J. Yu, S. Latour, A. Veillette, Clnk, a novel SLP-76-related adaptor molecule expressed in cytokine-stimulated hemopoietic cells, J. Exp. Med. 190 (1999) 1527–1534.
[3] R. Goitsuka, H. Kanazashi, H. Sasanuma, Y. Fujimura, Y. Hidaka, A. Tatsuno, A BASH/SLP-76-related adaptor protein MIST/Clnk involved in IgE receptor-mediated mast cell degranulation, Int. Immunol. 12 (2000) 573–580.
[4] H. Sasanuma, A. Tatsuno, K. Tsuji, S. Hidano, S. Morita, T. Kitamura, M. Kubo, D. Kitamura, R. Goitsuka, Transcriptional regulation of SLP-76 family hematopoietic cell adaptor MIST/Clnk by STAT5, Biochem. Biophys. Res. Commun. 321 (2004) 145–153.
[5] R. Goitsuka, A. Tatsuno, M. Ishiai, T. Kurosaki, D. Kitamura, MIST functions through distinct domains in immunoreceptor signaling in the presence and absence of LAT, J. Biol. Chem. 276 (2001), 36043–3650.
[6] Y. Fujii, S. Wakahara, T. Nakao, Targeting of MIST to Src-family kinases via SKAP55-SLAP-130 adaptor complex in mast cells, FEBS Lett. 540 (2003) 111–116.
[7] J. Yu, C. Riou, D. Davidson, Synergistic regulation of immunoreceptor signaling by SLP-76-related adaptor Clnk and serine/threonine protein kinase HPK-1, Mol. Cell. Biol. 21 (2001) 6102–6112.
[8] O. Utting, B.J. Sedgmen, T.H. Watts, X. Shi, R. Rottapel, A. Iulianella, Immune functions in mice lacking Clnk, a SLP-76-related adaptor expressed in a subset of immune cells, Mol. Cell. Biol. 24 (2004) 6067–6075.
[9] H. Sasanuma, A. Tatsuno, S. Hidano, K. Ohshima, Y. Matsuzaki, K. Hayashi, C.A. Lowell, D. Kitamura, R. Goitsuka, Dual function for the adaptor MIST in IFN-γ production by NK and CD4$^+$NKT cells regulated by the Src kinase Fgr, Blood 107 (2006) 3647–3655.
[10] Z.Q. Yang, W. Cheng, L.X. Hong, S.C. Lin, J.H. Han, H.M. Zhou, J. Gu, Adenine nucleotide (ADP/ATP) translocase 3 participates in the tumor necrosis factor-induced apoptosis of MCF-7 cells, Mol. Biol. Cell. 18 (2007) 4681–4689.
[11] J.Q. Li, Q.X. Li, C.C. Xie, H.M. Zhou, Y.Q. Wang, N. Zhang, H.J. Shao, S.C. Chan, X.X. Peng, S.C. Lin, J.H. Han, β-actin is required for mitochondria clustering and ROS generation in TNF-induced, caspase-independent cell death, J. Cell. Sci. 117 (2004) 4673–4680.
[12] P. Vandenabeele, L. Galluzzi, T. Vanden Berghe, G. Kroemer, Molecular mechanisms of necroptosis an ordered cellular explosion, Nat. Rev. Mol. Cell. Biol. 11 (2010) 700–714.
[13] D.W. Zhang, J. Shao, J. Lin, N. Zhang, B.J. Lu, S.C. Lin, M.Q. Dong, J. Han, RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis, Science 325 (2009) 332–336.
[14] J. Zhao, S. Jitkaew, Z. Cai, S. Choksi, Q. Li, J. Luo, Z.G. Liu, Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis, PNAS 109 (2012) 5322–5327.
[15] L. Sun, H. Wang, Z. Wang, S. He, S. Chen, D. Liao, L. Wang, J. Yan, W. Liu, X. Lei, X. Wang, Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase, Cell 148 (2012) 213–227.
[16] X. Chen, W. Li, J. Ren, D. Huang, W.T. He, Y. Song, C. Yang, W. Li, X. Zheng, P. Chen, J. Han, Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death, Cell. Res. 24 (2014) 105–121.
[17] Z. Cai, S. Jitkaew, J. Zhao, H.C. Chiang, S. Choksi, J. Liu, Y. Ward, L.G. Wu, Z.G. Liu, Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis, Nat. Cell. Biol. 16 (2014) 55–56.
[18] H. Wang, L. Sun, L. Su, J. Rizo, L. Liu, L.F. Wang, F.S. Wang, X. Wang, Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3, Mol. Cell. 54 (2014) 133–146.
[19] H.M. Zhou, L. Yang, H.J. Li, L.J. Li, J.M. Chen, Residues that affect human Argonaute2 concentration in cytoplasmic processing bodies, Biochem. Biophys. Res. Commun. 378 (2009) 620–624.
[20] A.L. Olins, G. Rhodes, D.B. Welch, M. Zwerger, D.E. Olins, Lamin B receptor: multi-tasking at the nuclear envelope, Nucleus 1 (2010) 53–70.