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# Necroptosis
## STAT3 kills?

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**TNF-induced necroptosis is caused by the activation of RIPK1 and the subsequent production of reactive oxygen species in the mitochondria, although the intermittent molecules of the signaling pathway responsible for this ROS-mediated type of programmed necrosis have not yet been identified. A recent article by Shulga and Pastorino in the *Journal of Cell Science* identifies RIPK1 as the mediator of STAT3 Ser727 phosphorylation, which leads to the translocation of the latter into the mitochondria via its interaction with GRIM-19, a member of the mitochondrial complex I. Here we discuss how the findings of the Shulga and Pastorino study shed light onto the involvement of STAT3 in necroptosis.**

**Keywords:** necroptosis, STAT3, RIP1, GRIM-19, ROS production

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The pleiotropic effects of the proinflammatory cytokine tumor necrosis factor (TNF) in activating various carious cell death pathways implicate TNF in the induction of a plethora of cellular processes, including cell death and survival, proliferation and differentiation.[1] In terms of cell survival or cell death pathways, TNF signaling results in nuclear factor kappa-B (NFκB) activation and survival, apoptosis or programmed necrosis, and in most cases these outcomes are mutually exclusive.[2] TNF can lead to the induction of either necrotic or apoptotic pathways via binding to the TNF-receptor 1 (TNFR1) that leads on to a cascade of events, the final outcome of which depends on the activation or inhibition of caspases. Although the fact that TNF can lead to either apoptosis or necrosis in different cell types has been known for some time,[3] the discovery that TNF can also induce a programmed form of necrosis, called necroptosis, was made more recently.[4,5] Inhibition of TNF- and Fas ligand (FasL)-induced apoptosis by Fas-associated protein with death domain (FADD), as well as deficiencies in caspase-8 and FADD (or inhibition of caspase-8 by the pan-caspase inhibitor zVAD or by viral proteins) result in necroptosis.[5,6]

Necroptosis requires the activation of the serine/threonine kinase receptor-interacting protein 1 (RIP1) and is defined as a type of cell death that is inhibited following ablation of RIP1[7-9] and this necroptotic kinase activity of RIP1 can be allosterically inhibited by necrostatins without affecting the kinase-independent, RIP1-mediated, activation of NFκB.[10] RIP1 along with RIP3, TRADD and FADD form the "necrosome" complex, which differs from the pro-apoptotic complex II in the lack of caspase-8.[11] Both RIP1 and the related RIP3 regulate their activation in the necroptotic process via auto- and trans-phosphorylation and their activity is required for the formation of the necrosome and the execution of necroptosis.[12,13] In several cell types, the production of reactive oxygen species (ROS) by the mitochondrial complex I is necessary for TNF-induced necrosis and is regulated by RIP1 and RIP3.[14,15] However, the downstream components that link the necrosome with the metabolic pathway have not been elucidated. The recent study by Shulga and Pastorino implicates the signal transducer and activator of transcription 3 (STAT3) in necroptosis via its known interaction with GRIM-19, a mitochondrial complex I component.[16] The Ser727 phosphorylation of STAT3 leads to its translocation to the

mitochondria, as opposed to Ser705 phosphorylation of STAT3 which leads to nuclear translocation. The STAT3 Ser727 phosphorylation and its mitochondrial translocation has now been found to be dependent on RIP1 activity since RNA silencing of RIP1 prevented both phosphorylation as well as the translocation of STAT3 to the mitochondria, which was associated with a reduction in ROS production.[16]

STAT3 is present in the mitochondria and its deletion leads to a decrease in the activity of mitochondrial complexes I and II, with reduced ROS production, indicating its importance in cellular metabolism.[17] The most recent data both confirm this metabolic role of STAT3 and additionally link STAT3 with necroptosis. This provides further evidence for a link between necroptosis and energy metabolism since STAT3 knockdown and expression of a non-phosphorylatable STAT3 Ser727 mutant resulted in failure of GRIM-19 mitochondrial translocation and inhibition of necroptosis. Similar results were obtained following reduction of GRIM-19 expression.[16] Furthermore, TNF-induced necroptotic death led to Ser727, but not Ser705, phosphorylation of STAT3 and an increase in ROS production.[16]

STAT3 has been implicated in cell death pathways and more specifically as an anti-apoptotic factor both in tumorigenesis as well as in the ischemic heart, where it acts as a cardioprotective agent.[18-20] The novelty of this study, therefore, is the finding that STAT3 can also function as a "pro-death" factor, albeit in a non-apoptotic manner. The study also suggests that RIP1 is a STAT3 kinase, though further work, such as in vitro kinase assays, is required to confirm this.[16]

The current study therefore raises some interesting points: First, small amounts of STAT3 are present in the mitochondria of various organs, although, apart from the present study, little is known about the role of phosphorylation (or, indeed, other post-translational modifications) in its bioenergetic activity.[17] STAT1 has also been detected in cardiac mitochondria, and ROS production in response to TNF-α has been reported to be reduced in livers from STAT1 knockout mice, although, again, the influence of STAT 1 post-translational modifications is unknown.[21,22] The interaction of STATs (whether singly, as homo- or heterodimers) with components of the electron transport chain (ETC) in modulating mitochondrial activity justifies more systematic investigation. Second, while Shulga and Pastorino found no evidence for STAT3 Tyr phosphorylation in necroptosis, does Tyr phosphorylation, with or without associated Ser phosphorylation, preferentially translocate STAT3 to the nucleus and away from the mitochondria? STAT3 phosphorylated only on the Ser residue can be a nuclear protein and therefore, does transcriptional activity contribute to the effects of STAT 3 on necroptosis? Third, what is the role of RIP3 in the STAT3/GRIM-19-mediated mitochondrial events during TNF-induced necroptosis? The homotypic interaction of RIP3 with RIP1 that leads to the formation of the necrosome renders RIP3 an important constituent of the necroptotic mechanism that is recruited by RIP1 but is also responsible for the phosphorylation of the latter.[12] Although it is known that RIP1 is involved in TNF-induced ROS production via recruitment of membrane-associated NADPH oxidase, the role of RIP3 in this process is still unknown.[11,23]

Therefore, this is a provocative study as well as one that presents some novel and unexpected observations. From the STAT3 perspective, in particular, it will be important to understand how the same protein can inhibit apoptosis yet promote necroptosis (**Fig. 1**). In a translational context, if we attempt to activate STAT3 as an inhibitor of apoptosis, for example, of neurons in stroke and of cardiomyocytes in myocardial infarction, will we simultaneously promote necroptosis with at best a neutral therapeutic outcome? Such considerations make the more detailed understanding of how STAT3 performs these antagonistic activities all the more necessary.

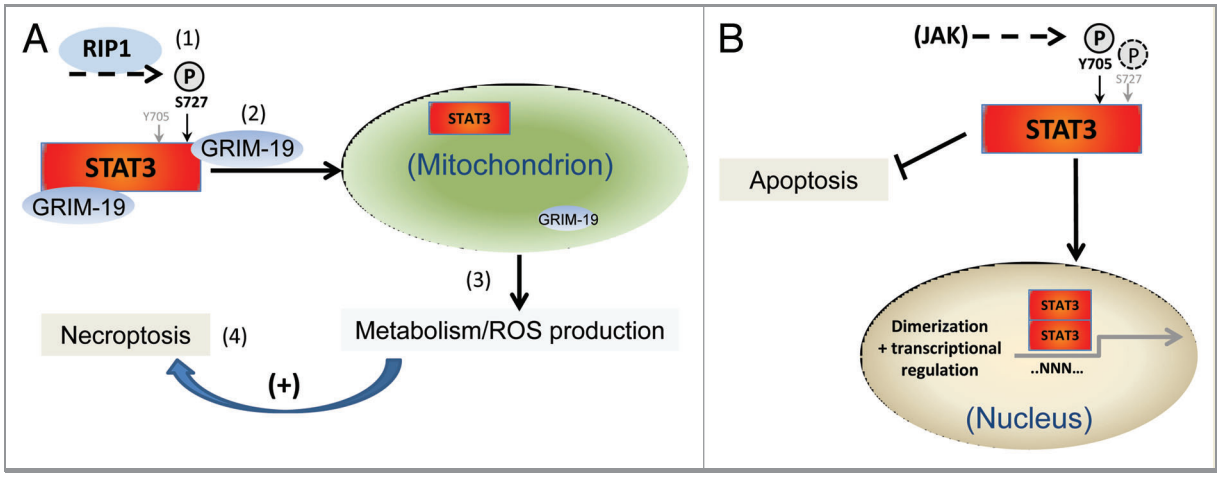


**Figure 1.** Model for a role of STAT3 in TNF-induced necroptosis according to the Shulga and Pastorino study. RIP1-mediated Ser727 phosphorylation of STAT3 (1) and its subsequent interaction with GRIM-19 (2) lead to the mitochondrial translocation of STAT3 and GRIM-19 into the mitochondria, where they elicit ROS production (3) and facilitate necroptosis (4) (A). Considering the new data, what are the key factors involved in STAT3 nuclear translocation and is this activated by Tyr705 phosphorylation alone or is Ser727 phosphorylation implicated as well? And how is the inhibitory effect of STAT3 on apoptosis linked to its induction of necroptosis (B)?

## References


1. Balkwill F. Tumour necrosis factor and cancer. Nat Rev Cancer 2009; 9:361-71; PMID:19343034; http://dx.doi.org/10.1038/nrc2628
2. Moquin D, Chan FK. The molecular regulation of programmed necrotic cell injury. Trends Biochem Sci 2010; 35:434-41; PMID:20346680; http://dx.doi.org/10.1016/j.tibs.2010.03.001
3. Laster SM, Wood JG, Gooding LR. Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J Immunol 1988; 141:2629-34; PMID:3171180
4. Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W, et al. Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. J Exp Med 1998; 187:1477-85; PMID:9565639; http://dx.doi.org/10.1084/jem.187.9.1477
5. Kawahara A, Ohsawa Y, Matsumura H, Uchiyama Y, Nagata S. Caspase-independent cell killing by Fas-associated protein with death domain. J Cell Biol 1998; 143:1353-60; PMID:9832562; http://dx.doi.org/10.1083/jcb.143.5.1353
6. Chan FK, Shisler J, Bixby JG, Felices M, Zheng L, Appel M, et al. A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. J Biol Chem 2003; 278:51613-21; PMID:14532286; http://dx.doi.org/10.1074/jbc.M305633200
7. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 2000; 1:489-95; PMID:11101870; http://dx.doi.org/10.1038/82732
8. Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, et al. Nomenclature Committee on Cell Death 2009. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 2009; 16:3-11; PMID:18846107; http://dx.doi.org/10.1038/cdd.2008.150
9. Lin Y, Choksi S, Shen HM, Yang QF, Hur GM, Kim YS, et al. Tumor necrosis factor-induced nonapoptotic cell death requires receptor-interacting protein-mediated cellular reactive oxygen species accumulation. J Biol Chem 2004; 279:10822-8; PMID:14701813; http://dx.doi.org/10.1074/jbc.M313141200
10. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, et al. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 2005; 1:112-9; PMID:16408008; http://dx.doi.org/10.1038/nchembio711
11. Vandenabeele P, Declercq W, Van Herreweghe F, Vanden Berghe T. The role of the kinases RIP1 and RIP3 in TNF-induced necrosis. Sci Signal 2010; 3:re4; PMID:20354226; http://dx.doi.org/10.1126/scisignal.3115re4
12. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 2009; 137:1112-23; PMID:19524513; http://dx.doi.org/10.1016/j.cell.2009.05.037
13. He S, Wang L, Miao L, Wang T, Du F, Zhao L, et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 2009; 137:1100-11; PMID:19524512; http://dx.doi.org/10.1016/j.cell.2009.05.021
14. Goossens V, Grooten J, De Vos K, Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proc Natl Acad Sci U S A 1995; 92:8115-9; PMID:7667254; http://dx.doi.org/10.1073/pnas.92.18.8115
15. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, et al. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 2009; 325:332-6; PMID:19498109; http://dx.doi.org/10.1126/science.1172308
16. Shulga N, Pastorino JG. GRIM-19 Mediated Translocation of STAT3 to Mitochondria is Necessary for TNF Induced Necroptosis. [Epub ahead of print]. J Cell Sci 2012; PMID:22393233; http://dx.doi.org/10.1242/jcs.103093
17. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, et al. Function of mitochondrial Stat3 in cellular respiration. Science 2009; 323:793-7; PMID:19131594; http://dx.doi.org/10.1126/science.1164551
18. Liu Y, Li P-K, Li C, Lin J. Inhibition of STAT3 signaling blocks the anti-apoptotic activity of IL-6 in human liver cancer cells. J Biol Chem 2010; 285:27429-39; PMID:20562100; http://dx.doi.org/10.1074/jbc.M110.142752
19. Jacoby JJ, Kalinowski A, Liu MG, Zhang SS, Gao Q, Chai GX, et al. Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, cardiac fibrosis, and heart failure with advanced age. Proc Natl Acad Sci U S A 2003; 100:12929-34; PMID:14566054; http://dx.doi.org/10.1073/pnas.2134694100
20. Hilfiker-Kleiner D, Hilfiker A, Fuchs M, Kaminski K, Schaefer A, Schieffer B, et al. Signal transducer and activator of transcription 3 is required for myocardial capillary growth, control of interstitial matrix deposition, and heart protection from ischemic injury. Circ Res 2004; 95:187-95; PMID:15192020; http://dx.doi.org/10.1161/01.RES.0000134921.50377.61
21. Lee HJ, Oh YK, Rhee M, Lim JY, Hwang JY, Park YS, et al. The role of STAT1/IRF-1 on synergistic ROS production and loss of mitochondrial transmembrane potential during hepatic cell death induced by LPS/d-GalN. J Mol Biol 2007; 369:967-84; PMID:17475277; http://dx.doi.org/10.1016/j.jmb.2007.03.072
22. Boengler K, Hilfiker-Kleiner D, Heusch G, Schulz R. Inhibition of permeability transition pore opening by mitochondrial STAT3 and its role in myocardial ischemia/reperfusion. Basic Res Cardiol 2010; 105:771-85; PMID:20960209; http://dx.doi.org/10.1007/s00395-010-0124-1
23. Kim YS, Morgan MJ, Choksi S, Liu ZG. TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell 2007; 26:675-87; PMID:17560373; http://dx.doi.org/10.1016/j.molcel.2007.04.021