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Cytoplasmic MYC is an anti-necroptotic protein

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ABSTRACT

Cancer cells are often resistant to necroptosis as well as apotosis, but the underlying mechanisms are not fully understood. We recently revealed an important crosstalk between MYC, a potent oncogene, and receptor-interacting protein kinase 3 (RIPK3), a pivotal factor in inducing necroptosis. Mechanistically, cytoplasmic MYC directly binds to RIPK3, inhibiting initial necrosome complex formation.

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Resisting cell death is a hallmark of cancer. While the mechanism by which cancer cells escape apoptosis is well-understood, little is known about how necroptosis is controlled by oncogenic signals in cancer cells.[1] Many types of cancer cells have protective mechanisms against necroptotic processes by developing systems that can negatively regulate factors involved in necroptosis such as receptor-interacting protein kinase 3 (RIPK3), possibly at the epigenetic, posttranscriptional, and posttranslational levels. These observations implicitly indicate that anti-necroptotic processes are selected in the evolution of cancer formation and offer the possibility of employing necroptosis to suppress tumorigenesis. However, unlike the targeting of apoptotic processes, targeting of the necroptotic process to stall cancer development has been tightly restricted by the lack of a comprehensive understanding of the crosstalk between the various tumor-promoting pathways and necroptosis, which paradoxically provides an uncharted field for exploration. We recently reported important and exciting findings of crosstalk and a regulatory pathway that exists between MYC, a potent oncogene, and RIPK3, a pivotal factor in inducing necroptosis.[2]

Necroptosis is a type of programmed necrotic cell death necrosis, which is activated by various inflammatory stimuli and DNA damage.[3] RIPK3 is a key protein in necroptosis execution, forming a complex with RIP homotypic interaction motif (RHIM)-containing proteins such as RIPK1.[4] Activated RIPK3 directly phosphorylates mixed lineage kinase domain-like protein (MLKL), which eventually destroys cellular membranes.[4] Of note, necroptosis is a mechanism that not only kills cells but also induces inflammation by releasing damage-associated molecular patterns (DAMPs) such as High Mobility Group Box 1 (HMGB1), interleukin-1α (IL-1α), ATP, and mitochondrial DNA (mtDNA) out of the cells, thereby boosting the inflammatory response of neighboring cells.[5] Therefore, necroptosis is regarded as immunogenic cell death (ICD), which is implicated in various inflammatory diseases and anti-cancer immunity.[6] Several studies have supported that cells dying by necroptosis strongly activate anti-cancer immunity.[7] Interestingly, cells dying by necroptosis undergo transcriptomic reprogramming to maximize the inflammatory response, as has been observed by our group and other researchers.[2] In contrast, we found MYC/MAX and E2F Transcription Factor 1 (E2F1) target genes are significantly downregulated in necroptotic cells, suggesting that oncogenic signaling pathways might be negative regulators of necroptosis. Indeed, MYC functions as a negative regulator of RIPK3 and prevents RIPK3-RIPK1 complex formation, leading to necroptosis suppression. The mechanism by which MYC suppresses necroptosis is quite unexpected because no transcriptional activity is required although MYC is a well-known transcription factor.[8] In addition, cytoplasmic MYC is sufficient to suppress necroptosis. A direct interaction and colocalization between MYC and RIPK3 plausibly takes place in the cytoplasm under normal conditions. This process seems to restrict RIPK3 from binding to RIPK1 and facilitates lysosomal degradation of RIPK1 and RIPK3 by STIP1 homology and U-Box containing protein 1 (STUB1), also known as carboxyl terminus of Hsp70-interacting protein (CHIP) (Figure 1). Interestingly, we observed a small complex consisting of RIPK3 and RIPK1 in MYC-depleted cells in the absence of necroptotic stimuli (Figure 1), supporting that the primary function of MYC is to prevent the spontaneous formation of RIPK1 and RIPK3. As a result, MYC ameliorates full necrosome formation and necroptotic cell death upon necroptotic stimuli. Given that RIPK3 is also localized in the nucleus upon necroptotic stimuli,[9] RIPK3 might interact with MYC in the nucleus. This interaction seems very transient because the nuclear colocalization between RIPK3 and MYC is only observed when nuclear export is inhibited. These data indicate that nuclear MYC might also contribute to necroptosis suppression.

The importance of MYC in preventing necroptosis becomes quite evident in the RIPK3 negative regulatory pathway, which upon activation by necroptotic stimulation, induces MYC degradation. Although RIPK3 kinase activity is required for

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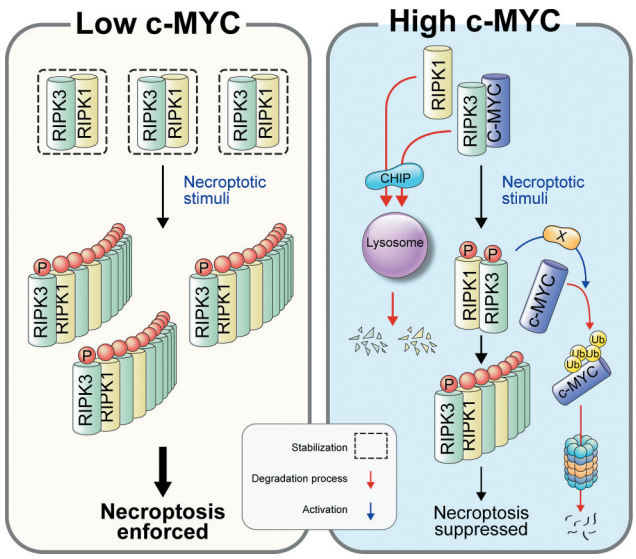

Figure 1. Mechanism by which MYC suppresses RIPK3-dependent necroptosis. Under normal conditions, cytoplasmic MYC directly binds to receptor-interacting protein kinase 3 (RIPK3), preventing RIPK1-RIPK3 complex formation. Free RIPK1 and RIPK3 undergo lysosomal degradation through ubiquitination by STIP1 homology and U-Box containing protein 1 (STUB1), also known as carboxyl terminus of Hsp70-interacting protein (CHIP). Upon necroptotic stimuli, RIPK1 and RIPK3 are phosphorylated and stabilized, leading to necroptosis. Meanwhile, RIPK3 induces proteasomal degradation of MYC, possibly by phosphorylating an unknown factor. When MYC is overexpressed, MYC suppresses initial RIPK1-RIPK3 complex formation, thereby inhibiting necroptosis. In contrast, when MYC levels are low, RIPK1 and RIPK3 spontaneously form a small complex in the absence of necroptotic stimuli, leading to its stabilization. Upon stimulation, the RIPK1-RIPK3 small complex contributes to the rapid formation of a large and insoluble necrosome complex, resulting in massive necroptotic cell death.

MYC degradation and RIPK3 is able to phosphorylate MYC *in vitro*, MYC phosphorylation by RIPK3 is not necessary for MYC degradation. Rather, we suggest the presence of other factors that are phosphorylated by RIPK3 for the degradation of MYC (Figure 1). In addition, we observed that the expression of MYC target genes is rapidly downregulated within 4 h of necroptotic stimuli, while MYC protein is degraded complete by 5 h. This observation suggests that nuclear RIPK3 might inhibit the transcriptional activities of MYC via direct binding or indirect phosphorylation of other proteins before inducing MYC degradation. Furthermore, MYC degradation might not be able to promote necroptosis because RIPK3 is already activated before MYC is degraded. Given that MYC also plays a suppressive role in inflammation.[7,10] early inhibition of MYC activity as well as MYC degradation can contribute to necroptosis-driven inflammatory responses.

Finally, we proposed the possibility that MYC suppression can stimulate necroptosis to induce cancer cell death in acute myeloid leukemia, which is characterized by overexpression of MYC as a positive selection factor for survival. We expect that this regulatory circuit of MYC and RIPK3 will provide a novel concept and perspective that is relevant for how necroptosis is regulated by a variety of oncogenic pathways. Although transcription factors such as MYC are regarded as undruggable targets, MYC is still an attractive target for cancer therapies in many ways. Beyond its classical roles in cell proliferation, MYC is regarded as the central factor for cancer metabolism, rendering cancer cells to survive under hypoxic and metabolic stress.[8] Recently, MYC was shown to directly transactivate CD47 (cluster of differentiation 47) and PD-L1 (programmed death-ligand 1), which abolish anti-tumor immunity.[10] Given that immunogenic cell death such as necroptosis strongly contributes to anti-tumor immunity, necroptosis suppression by MYC is thought to not only prevent cancer cells from dying but also to inhibitor anti-cancer immunity. New technology including proteolysis-targeting chimaera (PROTAC) might be used to develop promising MYC-targeting drugs.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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