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MYC needs MNT

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MYC family members *c-MYC*, *N-MYC* and *L-MYC* are amplified, translocated and otherwise deregulated in numerous and diverse tumor types. These events, which typically lead to constitutively increased MYC, provide a proliferative advantage to cells by boosting anabolic metabolism and supporting cell cycle progression.[1] However, the proliferative response to excessive MYC is countered in many cell types by increased apoptosis, and a number of studies have shown that events that inhibit MYC-driven apoptosis strongly cooperate with MYC in driving oncogenesis.[2]

High-level MYC expression, such as that found in many tumors, does occur normally in cells, but this typically happens only transiently in the context of growth factor-induced cell cycle entry. Cells are spared the proapoptotic effects of Myc in this setting, because mitogenic growth factors also stimulate prosurvival signaling.[3] Thus the apoptotic response to ectopic MYC likely results from a decoupling of increased MYC from prosurvival signaling needed for productive cell cycle entry and progression. But what are the key prosurvival factors and events that normally protect cells from the transient burst of MYC during cell cycle entry, and how might these events impinge on MYC-driven oncogenesis?

One factor that appears to protect cells from MYC-provoked apoptosis is MNT.[4] MNT shares with MYC a related basic-helix-loop-helix domain that mediates dimerization with MAX and DNA binding at E-box sequences, but MNT and MYC have opposing transcriptional activity.[4] Consistent with MNT functioning as a MYC antagonist, ectopic MNT expression can interfere with MYC-dependent transcription activation as well as proliferation and transformation of cells in culture.[4] And vice versa, MNT deficiency sensitizes cells to apoptosis but can also cause precocious cell cycle entry and lead to tumor formation in vivo.[4] Our recent studies indicate that MNT-deficient cells have increased sensitivity to apoptosis in the presence of ectopic MYC, but also upon mitogen-induced expression of endogenous MYC.[5] In the setting of Concanavalin A-stimulated cell cycle entry, where c-MYC is induced, proliferative expansion was dependent on MNT-mediated survival.[5] These results suggest that the prosurvival function of MNT plays a central role in the setting of cell cycle entry, where MYC induction is crucial (**Fig. 1**). Therefore, while premature cell cycle entry in the absence of MNT is linked to misregulation of MYC target genes involved in G_1-S transition,[4] it may also be that the antagonistic activities of MNT and MYC are involved in fine-tuning transcriptional programs that govern survival during cell cycle entry and progression.

Since MNT can antagonize both the pro-proliferative and pro-apoptotic activities of MYC, it was of interest to determine the role of MNT in MYC-driven tumorigenesis. Mice lacking *Mnt* are runted and die soon after birth.[6,7] Therefore, we used a conditional approach to simultaneously delete *Mnt* and induce ectopic expression of either wild-type c-MYC or the more oncogenic T58A mutant specifically in T cells.[5] Whereas the relatively small increase in wild-type c-MYC did not result in robust tumorigenesis, expression of the T58A mutant did. Importantly, simultaneous *Mnt* deletion and c-MYCT58A expression resulted in a significant increase in lifespan compared with c-MYCT58A mice (220 d vs. 355 d).[5] And while c-MYCT58A-expressing mice lacking MNT did not live a full lifespan, there was no evidence that tumor formation was responsible for their premature death.[5] We had previously shown that deletion of *Mnt* alone in T cells led to a lymphoproliferative disease that progressed to cancer in many mice.[8] Thus, whereas both forced MYC expression and *Mnt* deletion can be oncogenic, the combined proapoptotic tendencies of these individual conditions appear responsible for high levels of apoptosis that prevent tumorigenesis. Similarly, we found that MNT deficiency further sensitized MYC-overexpressing mouse embryo fibroblasts (MEFs) to apoptosis, and that the increased apoptosis could not be prevented by coexpression with oncogenic H-RAS or BCL2.[5] Moreover, MEFs expressing these strongly oncogenic protein combinations but lacking MNT were refractory to tumorigenesis.[5] These results raise the possibility that MNT might be broadly required for MYC-dependent oncogenesis.

Mnt-null cells were found to exhibit high levels of reactive oxygen species (ROS) and a super-sensitivity to drugs that inhibit antioxidant systems.[5] These results suggested that hyperproduction of ROS in *Mnt*-null cells might be why they are sensitized to apoptosis, either by drug inhibitors of antioxidant systems or by depletion of serum and glutamine.[5] Our results show that ROS were significantly higher in Mnt-deficient MEFs than in MEFs overexpressing c-MYC and thus support the idea that MNT normally prevents ROS production by more than simply inhibiting the activity of MYC. We postulate that MNT controls ROS production at least in part through unique activities involved in promoting efficient oxidative metabolism in mitochondria. For example, the reduced oxygen consumption in *Mnt*-null cells[5] might reflect a requirement for MNT to

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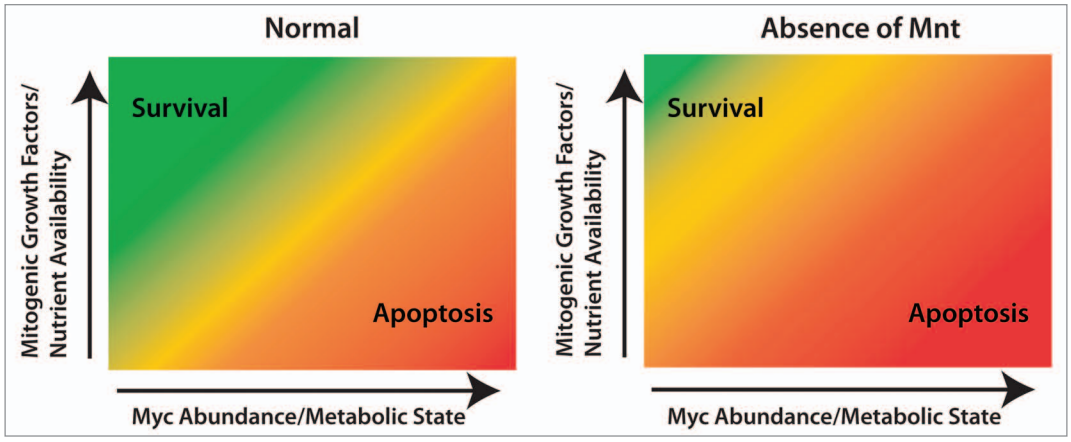

Figure 1. Loss of MNT reduces the threshold for MYC-dependent apoptosis. High levels of MYC can stimulate a hypermetabolic state and proliferation but will induce apoptosis (red zone) when not properly coupled to mitogenic stimulation and sufficient nutrient availability. Cells lacking MNT appear to not tolerate the hypermetabolic state induced by MYC and, as a result, exhibit a shift in the threshold at which they undergo apoptosis caused by forced MYC expression, induced MYC expression in the context of cell cycle entry and progression, or by treatment with inhibitors of antioxidant pathways.

adequately fuel oxidative phosphorylation in mitochondria or for the proper expression of proteins involved in mitochondrial metabolism. Given the positive effect MYC has on anabolic metabolism,[1] we hypothesize that MNT and MYC co-evolved to fine-tune gene expression programs that govern key metabolic changes required for efficient cell cycle entry and progression.

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