Endothelial cell killing by TAK1 inhibition: a novel anti-angiogenic strategy in cancer therapy

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Abstract

TAK1 is a key mediator of proinflammatory signals. In this issue of Developmental Cell, Naito *et al*. (2019) report that TAK1 loss from endothelial cells in adult mice results in intestinal and hepatic vascular destruction due to TNF-induced death of endothelial cells. Additionally, endothelial TAK1 deletion reduces tumor burden.

Main preview text

Tumor necrosis factor (TNF) is a proinflammatory cytokine that regulates cell death and inflammation. TNF is essential for immunity to certain pathogens, most importantly, *Mycobacterium tuberculosis*. It is becoming increasingly clear that imbalances in the signaling pathways regulated by TNF can lead to chronic inflammatory and autoimmune disorders as well as cancer. Understanding the mechanisms by which TNF regulates cell death and inflammation is therefore important to identify novel therapeutic means to tackle these diseases. In this issue of Developmental Cell, Naito *et al.* (2019) show that absence of the TGFβ-activated kinase 1 (TAK1) from endothelial cells (ECs) unleashes their TNF-mediated death, resulting in destruction of the normal vascular network in various tissues, most prominently in the gut and the liver.

When TNF binds to its ubiquitously expressed cognate receptor, TNFR1, the so-called TNFR1 signaling complex (TNFR1-SC), also referred to as complex I of TNFR1 signaling, is rapidly formed. The complex recruits, via different adaptors, the kinase RIPK1 and the ubiquitin ligases cIAP1, cIAP2 and LUBAC. Different types of ubiquitin chains placed on components of the complex promote recruitment and activation of various functional kinase-containing units, including the TAK1/TAB1/TAB2 kinase complex. The activities of all kinases associated with these different functional units, including that of TAK1, are required to keep TNF from inducing cell death via phosphorylation of RIPK1 in the TNFR1-SC, thereby preventing its release from this complex (Annibaldi and Meier, 2018) (Figure 1).

TNF-induced cell death is triggered by a secondary complex, referred to as complex II of TNFR1 signaling. Complex II forms when RIPK1 is released from complex I and recruits FADD, caspase-8, cFLIP and RIPK3. Caspase-8 activation can result in apoptosis, however, when caspase-8 is inactivated, RIPK1 can induce necroptosis via recruitment of RIPK3 and phosphorylation of MLKL which activates its necroptosis-inducing capacity (Annibaldi and Meier, 2018).

The study of mouse models lacking key components of the TNFR1-SC or of complex II have greatly contributed to our understanding of how TNF regulates cell death and inflammation. Curiously, deletion of essential LUBAC components, cIAP1/2 or TAK1 have all been shown to induce vascular defects resulting in embryonic death at mid-gestation (Morioka et al., 2012; Moulin et al., 2012; Peltzer et al., 2018). In the majority of cases, embryonic death was due to excessive cell death, mainly mediated by TNF. Embryonic lethality and loss of vascularization was also observed in mice deficient for caspase-8 or FADD. In this case,

lethality is mediated by aberrant induction of RIPK3-mediated necroptosis (Kaiser et al., 2011; Oberst et al., 2011). Curiously, however, although NF-κB-null mice also die during embryogenesis, they succumb at a later stage of gestation to apoptosis in the liver. Importantly, no aberrations in ECs or vasculogenesis were reported for these mice. Together, these findings imply that prevention of untoward TNF-mediated EC death, independently of NF-κB activation, is crucial to maintain their integrity during embryogenesis (Rudolph et al., 2000). Although the role of caspase-8, LUBAC and cIAPs in ECs is clearly cell death-dependent, the role of TAK1 in maintaining EC integrity has been less clear. Naito et al. (2019) provide important new insight on the role of TAK1 in ECs and report on how TAK1 inhibition may be harnessed for cancer therapy.

Naito et al. (2019) report that TAK1 deletion specifically from ECs in adult mice leads to death within six days. This is accompanied by complete destruction of vascular structures in the intestine and the liver, which is caused by aberrant cell death induction. The authors identified that TNF produced by myeloid cells in the intestine, which were stimulated by luminal bacteria, was the primary pathological event after TAK1 deletion. Inflammatory signals spread to the liver where residential macrophages produce TNF which kills TAK1-deficient ECs. Although intestine and liver are the organs that suffer most from EC-specific TAK1 deletion under physiological conditions and although dysfunction in these organs likely causes death of the animals, other organs are also affected. By performing two tissue injury models, the authors found that vascular destruction also takes place in the lungs and muscles under inflammatory conditions and, again, TNF produced by myeloid cells is responsible for EC death.

Naito et al. (2019) also investigated the effect of EC-specific deletion of TAK1 in subcutaneous tumor models which are known to be refractory to anti-VEGFR treatment. Loss of TAK1 from ECs effectively prevented tumor growth and even induced tumor regression. The obvious caveat of this model is that mice succumbed to TAK1 deletion in ECs. However, the authors also injected TAK1 inhibitor directly into the tumor and observed a similar reduction in tumor growth. Intriguingly, both EC death and vascular destruction are caused by TNF, which is also responsible for the antitumor activity of EC-specific TAK1 inhibition since ablation of TNFR1 prevented EC death, restored normal vascularization to gut and liver, and interfered with the antitumor activity of TAK1 inhibition.

Because many tumors are refractory to anti-VEGFR treatment, alternative strategies to tackle angiogenesis during tumorigenesis are needed. This work demonstrates that TAK1 inhibition could represent such an alternative strategy. To date, TAK1 inhibition has been suggested to act primarily by inducing apoptosis in tumor cells, e.g. in a KRAS-dependent *in-vivo* model of colon cancer (Singh et al., 2012). However, the *in-vivo* results provided by Naito et al. (2019) suggest that TAK1 inhibition may exert its anti-tumor effect, at least in part, in a tumor-cell non-intrinsic manner by enabling the TNF-induced death of ECs in the tumor microenvironment.

TAK1 inhibition is an attractive new therapeutic avenue, yet a number of questions remain to be addressed. Although it is now clear that TAK1 deficiency induces EC death, the precise mechanisms of cell death induction, most importantly including the mode of death by which the ECs die when TAK1 is absent or inhibited, have not been fully elucidated. In addition, TAK1 has also been implicated in non-cell death-inducing pathways related to angiogenic cell migration (Morioka et al., 2012). Lastly, although TNFR1 deletion significantly ameliorated vascular integrity and improved survival, it did not fully rescue the mice from premature lethality. Therefore, there are either alternative, TNF-independent, mediators of EC death or cell death-independent mechanisms regulated by TAK1 that are not to be

neglected. Future research aimed at revealing the function of TAK1 in tissue homeostasis will likely provide answers to these questions and, thereby, hopefully pave the way for the clinical application of TAK1 inhibition.

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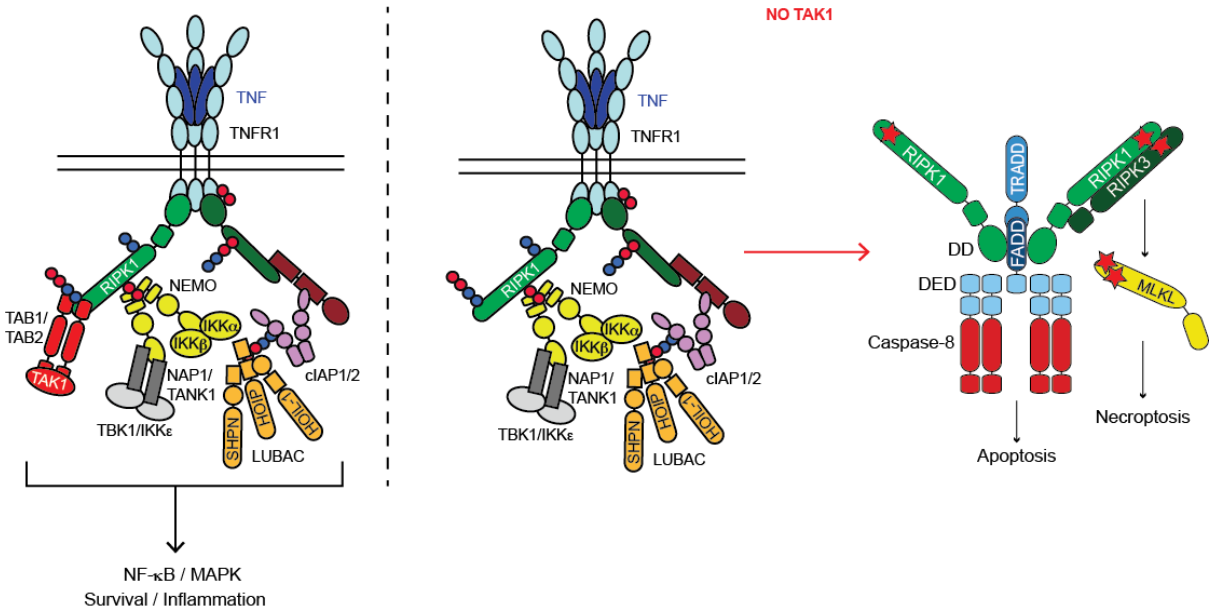

Figure 1: TAK1 regulates cell death and inflammation in TNFR1 signaling. Under physiological conditions, engagement of TNFR1 by TNF induces formation of the TNFR1 signaling complex (TNFR1-SC) in which the kinase-containing functional units TAK1/TAB1/2 and IKKα/β/NEMO respectively induce MAPK- and NF-κB-mediated expression of survival genes and proinflammatory cytokines (left panel). Apart from this activity, these two kinase complexes act together with the kinases TBK1 and IKKε (Lafont et al., 2018) to keep the kinase activity of RIPK1 in check. In the absence of TAK1 from endothelial cells, TNF kills them. Importantly, this function is independent of TAK1's role in NF-κB and MAPK-mediated gene activation, but instead due to formation of the cell death-inducing complex II of TNFR1 signaling (right panel) by the failure to keep RIPK1 in check in the TNFR1-SC when TAK1 is absent or inactive.