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Commentary:

Controlling inflammation: Miz1 and FBXW7 rein in C/EBPδ

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Inflammatory signaling is necessary both for innate and adaptive immune responses. Whereas innate immune responses are associated with physiological processes such as wound healing, chronic inflammation culminates in pathological conditions leading to aggravation of inflammatory diseases. Several reports have elegantly demonstrated the involvement of Toll-like receptors (TLRs), NF-kB and CCAAT/enhancer binding proteins (CEBPs) in the initiation and amplification of the inflammatory response (Litvak et al, 2010; Goldszmid and Trinchieri, 2012), however very little is known about how the inflammatory response is resolved, a process which is crucial for the benefit of the host.

A study by Do-Umehara et al (Nature Immunology, 2013) recently showed that Miz1 is a factor that is necessary to resolve inflammatory signaling. Miz1 is a member of the POZ domain/zinc finger transcription factor family known for its repressive function(s) together with its co-partner Myc (Herkert and Eilers, 2010). Previously, a non-transcriptional activity of Miz1 was reported whereby cytoplasmic Miz1 interfered with JNK activation leading to suppression of lipopolysaccharide (LPS)- and TNF-induced inflammatory responses (Liu et al, 2012). However, the role of nuclear Miz1 in inflammatory responses is not yet known. In this study, the authors used a lung-injury mouse model to investigate the role of the Miz1 POZ domain in inflammatory responses. The authors found that mice with a lung-specific deletion of the Miz1 POZ domain are highly susceptible to LPS-induced inflammation and acute lung injury. Primary alveolar type II (ATII) cells treated with LPS after infection with adenoviral vector constructs expressing Miz1(ΔPOZ) augmented the production of pro-inflammatory cytokines and chemokines compared to Miz1(WT). Next, the authors investigated the mechanistic details of how loss of the Miz1 POZ domain increased the pro-inflammatory signaling that culminate in severe lung injury. The authors ruled out that the hyper susceptibility of *Miz1*$^{\Delta POZ-lung}$ mice to LPS was caused by an alteration in the activation of MAP kinases or NF-κB. The authors then focused on C/EBP transcription factors which are crucial regulators of inflammatory signaling. Interestingly, disruption of Miz1

resulted in higher levels of *Cebpd* (C/EBPδ) mRNA and protein in primary lung ATII cells whereas other C/EBP family proteins were not affected. Indeed, silencing of C/EBPδ almost completely abolished the enhanced production of pro-inflammatory cytokines and chemokines in mouse MLE-12–Miz1(ΔPOZ) type II-like lung epithelial cells. The question then became: how does Miz1 regulate C/EBPδ expression? Promoter analysis demonstrated that Miz1 directly repressed *Cebpd* promoter activity via histone deacetylase 1(HDAC1). The authors further found that Miz1 controls binding of NF-kB and ATF-3 to the *Cebpd* promoter. These factors are known positive and negative regulators, respectively, of C/EBPδ transcription (Litvak et al, 2009). When they analyzed post-translational modifications of Miz1 in response to TNF-α that might cause the suppressive effect of Miz1, they found that Miz1 was phosphorylated at Ser178 after stimulation with TNF-α. Mutational analysis indicated that this Ser178 phosphorylation is necessary for Miz1's suppressive function on C/EBPδ expression. Altogether these findings indicate that phosphorylation of Miz1 and its cooperation with HDAC1 suppresses inflammation by inhibiting *Cebpd* expression.

While these findings add significantly to our understanding of the resolution of inflammation, the following questions remain to be answered in order to obtain complete mechanistic details by which Miz1 functions as a suppressor of inflammatory signaling. For instance, the authors show that serine phosphorylation of Miz1 is necessary for its recruitment to the *Cebpd* promoter. However, the kinase required for Miz1 activation in response to TNF-α is not known. Moreover, the question of whether TNF-α is necessary or sufficient to resolve inflammation remains unanswered. Furthermore, more studies are needed to clarify how Miz1 integrates into the NF-κB–ATF-3–C/EBP-δ transcription-regulatory circuit and to determine the mechanism how Miz1 regulates ATF-3 expression (although the authors have found that Miz1 modulates ATF-3 expression). Additionally, the Miz1 partner Myc, together with Max, has been shown to inhibit C/EBPδ expression in mammary epithelial cells (Si et al, 2010)- but the role of Myc and Max in the inflammatory regulation of *Cebpd* promoter activity in the context of inflammation remains to be determined.

Recently we have shown that C/EBPδ-mediated inflammatory signaling can be inhibited by the F-box protein FBXW7α which targets C/EBPδ protein for degradation and functions as an attenuator of inflammatory signaling (Balamurugan et al, 2013). The anti-inflammatory functions of Miz1 and FBXW7 can be explained by their role in the negative regulation of C/EBPδ expression. However, the question remains if

For example, is re-activation of FBXW7 observed after inhibition of *Cebpd* by Miz-1, and would this be necessary for sustained repression of inflammation by Miz1? It is possible that C/EBPδ expression under non-inflammatory conditions is predominantly regulated by FBXW7and that Miz1 "takes-charge" under inflammatory conditions (e.g. LPS and TNF-α). Together, these findings open up new avenues of investigation to address the mechanisms of activation of Miz1 and/or FBXW7 as potential strategies for the treatment of inflammatory diseases.

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