Sterile inflammation fuels gastric cancer

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Constitutively activated NF-κB signaling has long been known to be oncogenic. In this issue of Immunity, O'Reilly et al. (2018), unveil a link between loss of NF-κB1, aberrant STAT1 signaling, sterile inflammation and the increased expression of immune checkpoint molecules as cancer drivers.

The family of transcription factors referred to as nuclear factor kappa B (NF-κB) regulates the transcription of many genes involved in central physiological processes. These include, amongst others, cell proliferation, differentiation, survival and death as well as inflammation and the overall orchestration of the immune response. In addition, dysregulated NF-κB signaling has long been known to exert functions in autoimmunity (Sun et al., 2013) and tumorigenesis, particularly the development of inflammation-associated cancers, such as chemical injury-induced development of liver cancer (Karin, 2009). However, the precise role of the various members of the NF-κB family in tumorigenesis is largely unknown. In this issue of Immunity, O'Reilly et al. (2018) elegantly demonstrate a role for NF-kB1 in tumor suppression.

The family of protein dimers referred to as NF-κB are formed by the combination of members of the so-called reticuloendotheliosis (Rel) protein family. The members of this family are characterized by a Rel homology domain, which confers the ability to dimerize and to bind DNA. Based on structure, function and biosynthesis, the REL protein family can be classified into two distinct groups. The first group comprises of RelA (also known as p65), RelB and cRel, which have intrinsic transcriptional transactivation function and do not require proteolytic processing, as they are synthesized in the mature form. The second group consists of NF-κB1 (also known as p105) and NF-κB2 (also known as p100), which lack intrinsic transcriptional transactivation function and require proteolytic processing to generate the mature p50 and p52 proteins, respectively (Karin et al., 2002). Whereas RelA, RelB and cRel contain a transactivation domain, which is essential for their ability to induce gene expression, this domain is absent from p50 and p52. Thus, some of the NF-κB dimers, such as the NF-κB1 (p50/p50) homodimers, lack intrinsic transcriptional trans-activation capacity, and as homo-dimers can act as inactive or repressive complexes unless the ability to act as a transcription factor is afforded to them via the association with other factors capable of coactivator recruitment (Smale, 2012). Several inflammatory stimuli that engage the IκB kinase complex allow NF-κB dimers, such as RelA/p50 heterodimers, to translocate to the nucleus and to initiate transcription of target genes (Karin, 2009).

In this issue of Immunity, O'Reilly et al. (2018) uncover a role of the NF-κB family member NF-κB1 as a crucial tumor suppressor in gastric cancer. Using NF-κB1-deficient mice the authors found that from 6 months of age, the mice start to develop diffuse gastritis and by 18 months, the majority of NF-κB1-deficient mice showed invasive gastric adenocarcinoma lesions. Accordingly, mice deficient in NF-κB1 presented characteristic features of gastric cancer, such as loss of the secreted Muc5AC mucin and increased levels of the surface-associated mucin Muc4. Dysregulation in the expression of these mucins has been associated with tumor invasion and proliferation in gastric cancer. Since the association between inflammation and cancer is well established, O'Reilly et al. (2018) examined whether deficiency in NF-κB1 would impact not only the epithelial cells but also the hematopoietic compartment. They found that immune cell infiltration precedes disease onset, as NF-κB1-deficient mice from 3 to 5 months of age presented with high infiltration of both lymphoid and myeloid cells in their stomachs. By 16 to 20 months, the mice displayed not only marked immune cell infiltration in their stomach, but also enhanced expression of pro-inflammatory factors, such as Cyclooxygenase-2, metalloproteases and osteopontin, which are associated with advanced intestinal-type gastric cancer in humans. Bone marrow chimera experiments were used to determine the contribution of loss of NF-κB1 from the immune versus non-immune compartments to gastric cancer development. Interestingly, only the reconstitution of NF-κB1-deficient mice with NF-κB1-deficient bone marrow cells recapitulated all the features of gastric cancer progression observed in NF-κB1-deficient mice, suggesting that NF-κB1 deficiency in both compartments is necessary to cause gastric cancer.

Helicobacter pylori and high salt diet are two risk factors that are commonly associated with gastric cancer in humans (Krejs, 2010). In a comprehensive effort, O'Reilly et al. (2018) therefore next assessed whether either of these insults may also be involved in gastric carcinogenesis in NF-κB1-deficient mice. However, neither infection by *H. pylori* nor high salt diet accelerated the development of gastric cancer in NF-κB1-deficient mice. Notably, these mice developed gastric cancer with a histopathological progression that recapitulated the human disease even when kept in an abiotic environment, implying that sterile inflammation driven by loss of NF-κB1 can promote gastric cancer development. This is an unsuspected etiology for this type of cancer that may also contribute, possibly together with non-sterile inflammation, to the development of other cancers. Interestingly, NF-κB1 haploinsufficiency was sufficient to drive the development of gastric cancer, as demonstrated in experiments using Nfkb1 heterozygous mice. In human cancers, polymorphisms in the promoter region of the *NFKB1* gene have been associated with increased risk of certain epithelial cancers (Karban et al., 2004), further underlining the relevance of the findings by O'Reilly et al. (2018) for human cancer.

Finally, O'Reilly et al. (2018) investigated the mechanism by which loss of NF-κB1 from both epithelial and immune cells drives gastric cancer initiation and progression. Gene expression analysis revealed that several signaling pathways involved in inflammation, antigen presentation and immune checkpoints were dysregulated in both cancer cells and the immune cells in the tumor microenvironment. Activation of NF-κB and other inflammatory transcription factors, such as STAT, regulate the expression of many pro-inflammatory genes that have been associated with cancer progression (Karin, 2009). Intriguingly, many of the genes that were found to be dysregulated in NF-κB1-deficient mice were under the control of the JAK/STAT signaling pathway. The authors used two complementary approaches to address whether aberrant activation of JAK/STAT signaling was indeed responsible for the development of gastric cancer in these mice. First, O'Reilly et al. (2018) generated NF-κB1-deficient mice also carrying the $Gp130^{Y757}$ mutation which causes STAT1 and STAT3 hyper-activation. They found that these mice developed gastritis and invasive cancer at a considerably younger age than the NF-κB1-deficient animals. Next, the authors created NF-κB1-

deficient mice that were, in addition, heterozygous for Stat3 loss (because complete loss of Stat3 is embryonically lethal) as well as NF-κB1-deficient mice in which Stat1 was either heterozygously and homozygously lost. They found that loss of STAT3 did not prevent the formation of gastric adenocarcinoma lesions whereas the complete loss of STAT1 markedly inhibited gastric cancer development in NF-κB1-deficient mice. Taken together, O'Reilly et al. (2018) provide compelling evidence that STAT1 activation cooperates with NFκB1 loss to drive the development of gastric cancer.

The production of proinflammatory cytokines in the tumor microenvironment can affect several mechanisms of tumor progression including the recruitment and accumulation of immune cells that can support tumor progression directly, or alternatively, suppress ant-tumor immunity. In addition, these cytokines can act on the tumor itself, fostering cancer cell proliferation, invasion and metastasis. As an example, interleukin (IL)-1 receptor blockade is known to inhibit the development of gastric inflammation and carcinogenesis (Tu et al., 2008). O'Reilly et al. (2018) found that loss of NF-κB1 increased the expression of programmed cell death-ligand 1 (PD-L1) in myeloid and gastric epithelial cells, as well as of cytotoxic T lymphocyte-associated protein 4 (CTLA4) and programmed cell death-1 (PD-1) in the lymphoid compartment (Figure 1). Importantly, the increased expression of PD-L1 on myeloid cells was observed already at an early stage of the disease in NF-κB1-deficient mice. These immune checkpoints are responsible for the inhibition of crucial T cell effector functions and the instigation of the pro-tumorigenic microenvironment that drives cancer progression. Collectively, the findings presented by O'Reilly et al. (2018) show that loss of NF-κB1 and the consequent aberrant STAT1 activation conspire to drive sterile inflammation, dysregulation of immune checkpoints and, ultimately, the development of gastric cancer.

Restoring and boosting the immune system function are amongst the principal aims of cancer immunotherapy. Numerous clinical trials employing anti-CTLA4 and PD-1/PD-L1 inhibitors have shown a significant improvement in the survival outcome in several cancers (Ott et al., 2017). PD-1/PD-L1 inhibitors are currently under clinical evaluation in patients with advanced gastric cancer (Long et al., 2017). In the light of the findings by O'Reilly et al. (2018), it will not only be interesting to determine how patients with gastric cancer will respond to immune checkpoint blockade overall but also whether mutations in the gene NF-κB1 may correlate with response to immune checkpoint blockade. In the future it will also be interesting to explore whether such therapies may also be advantageous in patients with early stage gastric cancer and whether blockade of certain cytokines or inhibitors of JAK/STAT signaling will synergize with immune checkpoint blockade in patients with gastric cancer as the concomitant targeting of proinflammatory pathways and immune-regulatory signaling might be able to abrogate the protumorigenic crosstalk between cancer and immune cells.

Acknowledgements

The authors were supported by a Cancer Research UK (CRUK) programme grant (A17341), a Wellcome Trust Senior Investigator Award (096831/Z/11/Z), a European Research Council (ERC) advanced grant (294880) held by H.W., a CRUK Centre grant (515818), a CRUK Centre Network Accelerator Award on Cancer Immunotherapy (CITA) (525877), the Manchester–UCL CRUK Lung Cancer Centre of Excellence (522434), and the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

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Figure 1. Loss of NF-κB1 drives gastric cancer development through aberrant STAT1 activation and sterile inflammation.

Absence of NF-κB1 in both gastric epithelial cells and immune cells induces aberrant STAT1 activation, promoting the instauration of a protumorigenic microenvironment through sterile inflammation. NF-κB1-deficient epithelial cells express increased levels of major histocompatibility complex (MHC) class I and class II and of the immune checkpoint molecule programmed cell death-ligand 1 (PD-L1) which impairs tumor immune surveillance. Elevated levels of MHC class I-II and PD-L1 are also observed in myeloid cells deficient for NF-κB1 and upregulation of programmed cell death-1 (PD-1) is also induced in T cells deficient for NF-κB1 which further support tumor progression. Moreover, loss of NF-κB1 promotes accumulation of natural killer (NK) cells and release of several cytokines in the gastric cancer lesion. New therapeutic interventions proposed by O'Reilly et al. (2018) are: JAK/STAT inhibition, anti-PD-1/PD-L1, cytokine inhibition (as indicated), or their combination.