AIMing 2 Curtail Cancer

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The dysregulation of the relationship between gut microbiota and innate immune homeostasis can lead to a range of complex diseases. In this issue, Man et al. reveal that the intracellular innate sensor AIM2 regulates microbial and stem cell homeostasis in the gut to protect against colorectal cancer.

"Cancer is a word, not a sentence." These are the inspiring words of John Diamond, the British broadcaster and journalist who succumbed to cancer in 2001. In his memoir, a witty and moving account of his fight with cancer he wrote, "What can the chances be of any organ doing anything a billion and a half times and never making a mistake?" He questions what keeps our myriad of incessantly replicating cells from joining together in a state of "cellular anarchy" that can cause cancer. Although the answer to this question remains a complex multifactorial puzzle, the past few years have seen a surge in studies exploring the intimate link between the innate immune system, microbiota, and cancer. In this context, Man et al. (2015) (this issue of *Cell*) now identify a role for the innate immune sensor Absent in Melanoma 2 (AIM2) in inhibiting the development of colon cancer by controlling intestinal stem cell proliferation and regulating the gut microbiota.

Colon cancer is the second leading cause of cancer death in the United States. However, the underlying mechanisms that either predispose individuals to or promote development of colorectal cancer remain poorly understood. Aberrant expression of several innate immune sensors, including Toll-like receptors (TLRs) and NOD-like receptors (NLRs), is highly associated with cancers at barrier sites, particularly the intestinal mucosa (Luddy et al., 2014). In particular, NLRC4, NLRP3, NLRP6, and NLRP12 have been implicated in protection against colitis-associated colorectal cancer (CAC) by preserving the integrity of the epithelial barrier, checking altered cell signaling, and/or regulating the composition of microbiota colonizing the intestine (Janowski et al., 2013). Although protective in some circumstances, activation of innate sensors—particularly TLR4—can have detrimental effects. Indeed, the role of innate immunity in colorectal cancer stems from the recognition that inflammation is a major driver of carcinogenesis. Pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 are released following activation of the innate immune response and are linked to neoplastic transformation in CAC. Additionally, several NLRs are heterogeneous in their expression and can regulate diverse biological processes beyond inflammation and tissue homeostasis to include autophagy, transcription, and cellular development (Kufer and Sansonetti, 2011), suggesting that, in the context of a complex disease like cancer, innate sensors may exert multiple and disparate or combinatorial, cell-type-specific effects.

AIM2 was initially characterized as a gene that is upregulated upon melanoma tumor reversion (DeYoung et al., 1997). Subsequent in vitro work identified a protective role for AIM2 in breast and colon cancer (Patsos et al., 2010). AIM2 became an area of intense research focus after its recognition as a double-stranded DNA sensor in the host cell cytosol, capable of forming an oligomeric signaling complex called the inflammasome that activates caspase-1, leading to release of the pro-inflammatory cytokines IL-1β and IL-18 (Fernandes-Alnemri et al., 2009; Hornung et al., 2009). Clinically, the absence of AIM2 is associated with tumorigenesis; colon cancer patients exhibit reduced expression of *Aim2*, and lower expression correlates with a poor prognosis (Dihlmann et al., 2014). To gain insight into the role of AIM2 in colon cancer, Man et al. (2015) employ a model of azoxymethane (AOM) and dextran sulfate sodium (DSS)-induced colorectal cancer in WT and $Aim2^{-/-}$ mice. $Aim2^{-/-}$ animals exhibit greater tumor burden yet no difference in inflammasome-dependent cytokines at day 14 and day 80 post-exposure to AOM and DSS. $Caspase\text{-}1^{-/-}$ and $Asc^{-/-}$ mice exhibit a significantly reduced inflammatory response, suggesting that tumor progression mediated by lack of AIM2 is independent of its role in inflammasome activation. Whether AIM2- or NLR-mediated activation of the inflammasome is involved in limiting initial tumor formation remains open to question.

Irrespective of its role in inflammasome activation, previous reports have identified a role for AIM2 in inducing cell-cycle arrest in colon cancer cells (Patsos et al., 2010). Man et al. (2015) demonstrate that AIM2 exerts its function by inhibiting cellular proliferation, as the colons of $Aim2^{-/-}$ mice exhibit greater numbers of $Ki67^{+}$ and $BrdU^{+}$ cells and a global upregulation of proliferation-associated genes. $Aim2^{-/-}$ colons also contained higher levels of activated AKT, a cell survival factor, and inactivated PTEN, a tumor suppressor that negatively regulates the AKT pathway (Figure 1). This correlates with increased expression of the proto-oncogene c-Myc that promotes cell proliferation and transformation. Collectively, these data suggest that AIM2 may itself act as a tumor suppressor that, via yet-unknown mechanism(s), limits tumor cell proliferation by repressing proto-oncogenes and the AKT pathway. Loss of AIM2 also restricts the activation of pro-apoptotic factors caspase-3 and caspase-7 to limit cell death that may in turn contribute to increased cell proliferation (Figure 1).

AIM2, like many innate sensors, is fairly ubiquitous within host cells. Man et al. (2015) show that, in the context of regulating colorectal cancer, AIM2 functions mainly within the non-hematopoietic compartment but also to some degree within the hematopoietic compartment. The mechanism by which hematopoietic AIM2 contributes to colorectal tumorigenesis remains undefined, but it is reasonable to hypothesize that AIM2 has distinct functional roles in the hematopoietic and non-hematopoietic niche and possibly varied roles in different cell types constituting these niches. Previous work has demonstrated that intestinal stem cells are the cells of origin for intestinal cancers (Barker et al., 2009) and are particularly vulnerable to tumors induced by activating mutations in β-catenin. Using *Aim2*$^{-/-}$ mice expressing tamoxifen-inducible, aberrant Wnt/β-catenin signaling in Prom1^{+} stem cells, Man et al. (2015) demonstrate that AIM2 limits Prom1^{+} stem cell proliferation. Compared to WT mice, *Aim2*$^{-/-}$ mice have increased numbers of Prom1^{+} cells in the intestine that correlate with increased activation of AKT and expression of Ki67 and c-Myc (Figure 1). Modest cellular proliferation is also observed in wild-type (WT) mice, which may correspond with downregulation of *Aim2* following exposure to carcinogens. Further research is required to elucidate how *Aim2* is downregulated in the intestine during development of cancer. The possibilities are various, ranging from direct or indirect transcriptional inhibition of AIM2 by a putative inhibitor to regulation of RNA stability by non-coding RNAs. The gut microenvironment is complex, and it is likely that combinatorial signaling cascades triggered by both microbiota-derived and endogenous ligands trigger this downregulation.

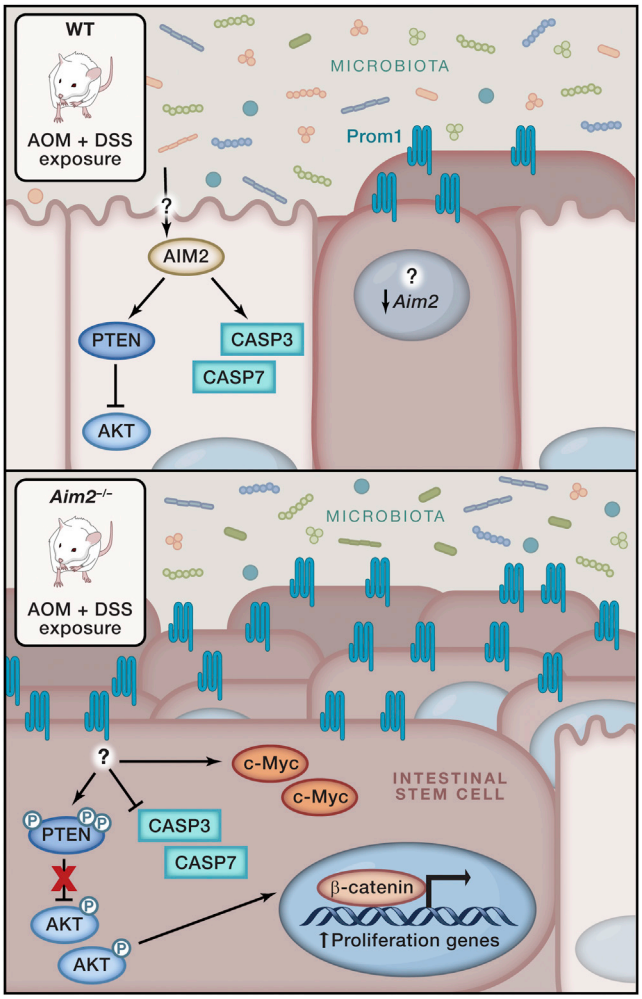

Figure 1. Role of AIM2 in Protection against Tumorigenesis

Decreased expression of the gene encoding AIM2 is linked to increased tumor growth and mortality in colorectal cancer patients. In a colitis-associated tumorigenesis model involving exposure to azoxymethane (AOM) and dextran sulfate sodium (DSS), WT mice (top) show decreased expression of *Aim2* and modest tumor growth in the colon. This is regulated by restriction of the AKT pathway by the tumor suppressor PTEN and increased activation of pro-apoptotic factors CASP3 and CASP7. In contrast, *Aim2*$^{-/-}$ mice (bottom) show rapid tumor growth promoted by expansion of Prom1^{+} intestinal stem cells in response to aberrant Wnt/β-catenin signaling. Through yet-unknown attributes, the loss of AIM2 results in the inactivation of PTEN via its phosphorylation, leading to activation (phosphorylation) of AKT and increased expression of genes involved in cell proliferation and tumorigenesis, including c-Myc. *Aim2*$^{-/-}$ mice also exhibit a modified microbiota composed of species linked to increased tumor development, further amplifying their susceptibility to colorectal tumorigenesis. Altered homeostatic microbiota and aberrant AKT and β-catenin signaling may thus act in concert to promote tumorigenesis resulting from loss of AIM2.

Intestinal cells engage with gut microbiota in an intimate crosstalk that is believed to regulate inflammation, cell proliferation, and development. Therefore, Man et al. (2015) further investigate the role of microbiota in the susceptibility of *Aim2*$^{-/-}$ mice to tumorigenesis. Interestingly, *Aim2*$^{-/-}$ mice exhibit an altered microbiota, containing bacterial species previously linked to colon cancer. Co-housing WT and *Aim2*$^{-/-}$ mice decreased the tumor burden in *Aim2*$^{-/-}$ animals, an observation that raises the possibility of microbiota engraftment as a preventive measure for reducing the risk of developing CAC resulting from loss-of-function mutations in AIM2. Of interest and yet to be studied is the causal relationship between lack of AIM2, increased cellular proliferation, and dysbiotic gut microbiota; nevertheless, the loss of AIM2 appears to elicit intrinsic immune mechanisms that aid in establishment of a microenvironment permissible to carcinogen-induced tumorigenesis. Availability of modified ligands such as microbe-associated molecular patterns and metabolites produced by gut microbiota or altered tumor antigens and danger signals released from host cells may further contribute to aberrant cell signaling and tumorigenesis in the *Aim2*$^{-/-}$ environment compared to the WT environment. Man et al. (2015) observe that tumor burden is modestly increased in WT mice co-housed with *Aim2*$^{-/-}$ mice when compared to singly-housed WT mice, indicating that dysregulation of the microbiota, at least in part, contributes to increased cellular proliferation and overall suggesting a complex link between the composition of gut microbiota and dysregulation of cellular homeostasis leading to tumorigenesis.

Although it is tentative to suggest modulation of AIM2 as a treatment for colorectal cancer, it must be done with caution. Most innate sensors with

recognized roles in regulation of carcinogenesis function as a double-edged sword, and AIM2 is no exception. Data presented here by Man et al. (2015) suggest that AIM2 is necessary to inhibit cellular, particularly intestinal stem cell, proliferation in response to carcinogens. Yet, overexpression of AIM2 can lead to increased cellular adhesion and invasiveness, which may promote metastasis (Patsos et al., 2010). Therefore, any modulation of *Aim2* expression must be tightly regulated.

Collectively, the intriguing new insights offered by Man et al. (2015) group AIM2 with a growing class of colorectal-cancer-associated immune sensors (Janowski et al., 2013). Based on their findings, interrogating how AIM2 acts in concert with other innate sensors such as NLRP3, NLRC4, NLRP6, and NLRP12 to control colorectal cancer may be the next step forward toward modulation of the innate immune system for therapeutic benefit. Nevertheless, in humans, the underlying heterogeneity and inherent nature of cancer as a multifactorial condition in which genetics and environment impinge upon each other to manifest a disease that is essentially "unique" from individual to individual poses a major challenge for cancer research. Cancer is an emergent property of the dysregulation of multiple epigenetic, transcriptional, molecular, and cellular circuits rather than the result of a single genetic event. Examining these multiple scales may enable a holistic understanding of the underlying factors and/or mechanisms that promote cancer. The road is long, but hopefully through relentless research efforts, literal meaning may be imparted to John Diamond's words—reducing cancer to a word that is no longer perceived as a "sentence."

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Brain Wiring in the Fourth Dimension

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In this issue of *Cell*, Langen et al. use time-lapse multiphoton microscopy to show how *Drosophila* photoreceptor growth cones find their targets. Based on the observed dynamics, they develop a simple developmental algorithm recapitulating the highly complex connectivity pattern of these neurons, suggesting a basic framework for establishing wiring specificity.

Large-scale efforts to precisely reconstruct the connectomes of different visual systems are uncovering a remarkable level of complexity. How this elaborate and precise wiring is established is a critical question, since the sheer number of specific connections presents a major wiring challenge. Design principles common between vertebrate and insect visual systems suggest that basic mechanisms for establishing wiring specificity may be shared between such distantly related species (Sanes and Zipursky, 2010). Using high-resolution time-lapse imaging and mathematical modeling of fly visual system neurons, Langen et al. (2015) (this issue of *Cell*) define a set of simple rules that are sufficient for wiring specificity of these neurons. Hence, a complex interplay of many specific guidance signals may not always be needed to establish precise connectivity.

The *Drosophila* visual system manifests a complex connectivity pattern of photoreceptor axons in the optic lobe and has long served as a model for how individual neurons find their appropriate synaptic partners (Hadjieconomou et al., 2011). The six outer photoreceptor neurons (R1–6) in each ommatidial unit