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Common TLR5 Mutations Control Cancer Progression

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The mechanisms regulating tumor-associated inflammation are incompletely understood. In this issue of *Cancer Cell*, Rutkowski and colleagues indicate that TLR5 signaling deficiency, which occurs in ~10% of the population, changes interactions with commensal microbiota and deregulates a cascade of inflammatory events that can suppress or accelerate extraintestinal cancers.

Cancer and inflammation were first linked more than 150 years ago by Robert Virchow, who observed that leukocytes frequently infiltrate tumors. Yet, the role of the immune system in malignancy has only recently come under more extensive study. At present, we understand that distinct immune components, which define a so-called immune contexture (Fridman et al., 2012), can either repress or promote cancer. Recent evidence also indicates that intestinal commensal bacteria profoundly influence immune cell activity in health and disease (Hooper et al., 2012) and modulate disease-associated immune responses to therapy even at extraintestinal sites (Iida et al., 2013; Viaud et al., 2013). Therefore, uncovering which microbiota-host interactions cause or prevent disease should increase our understanding of cancer and possibly offer new vantage points for therapy.

In this issue of *Cancer Cell*, Rutkowski et al. (2015) report that Toll-like receptor 5 (TLR5), a pattern recognition receptor that recognizes bacteria-derived products, can greatly affect extraintestinal tumor progression through differential modulation of systemic inflammation and tumor-associated immunity. The findings presented in this study might have broad consequences, because TLR5 loss-of-function mutations are frequent in humans (Casanova et al., 2011).

Using a conditional genetic mouse model of sarcoma driven by *Kras* and *Trp53* alterations, Rutkowski et al. (2015) initially found that TLR5 deficiency decreased systemic interleukin 6 (IL-6) serum levels and delayed tumor progression. In this model IL-6 also controlled myeloid cell mobilization, which is associated with accelerated tumor progression, according to previous studies (Gabrilovich et al., 2012). Interestingly, depleting commensal microbiota by orally administering antibiotics to TLR5-responsive mice was sufficient to control IL-6 levels, myeloid cell mobilization, and sarcoma outgrowth. Bacterial depletion also prevented accumulation of tumor-infiltrating γδ T cells, which can mediate immunosuppressive functions and promote cancer progression (Peng et al., 2007). Separate experiments involving antibody-mediated depletion or adoptive transfer of γδ T cells further indicated that the presence of this T cell subset accelerates malignant progression in TLR5-responsive hosts. These data show that TLR5-mediated host recognition of commensal bacteria can regulate tumor progression by affecting the tumor's immune contexture.

Rutkowski and colleagues further report that, in the presence of commensal bacteria, γδ T cells produce the immunosuppressive molecule galectin-1 (Rubinstein et al., 2004), thereby suggesting that microbiota also affect the functions of tumor-associated immune cells. Mechanistic studies indicate that myeloid cells can produce adenosine, which helps upregulate galectin-1 expression in γδ T cells. Also, by generating chimeras with γδ- and galectin-1-deficient bone marrow, the authors found that galectin-1 secreted by γδ T cells is sufficient to accelerate *Kras/p53*-driven sarcoma growth in mice.

Rutkowski et al. (2015) went on to assess consequences of TLR5 loss-of-function in mouse models of other extraintestinal cancers and found that TLR5 deficiency does not always suppress tumor growth. In fact, they determined that the A7C11 breast carcinoma cell line grows even faster in TLR5-deficient hosts than in their wild-type counterparts. The precise mechanisms that dictate whether TLR5 signaling will promote or suppress cancer require further study, yet initial findings indicate that cytokines can play a role. In TLR5-sufficient mice, several tumors (e.g., the MPKAS cell line derived from axillary tumors of the genetic *Kras/Trp53* mouse model and orthotopic ovarian cancers) increased systemic IL-6 serum levels and likely depended on IL-6 to grow, whereas A7C11 breast tumors did not. Instead, A7C11-bearing mice showed increased IL-17 serum levels in TLR5-deficient mice. Administering IL-17 blocking antibodies controlled A7C11 tumor progression. Also, commensal bacteria depletion in these mice reduced IL-17 levels and controlled tumor growth. Administering IL-17 blocking antibodies also controlled MPKAS tumor growth, but only when IL-6 was also blocked.

Taken together, the murine data suggest that IL-6 and IL-17 differ in their ability to influence tumor-promoting inflammation; IL-6 may be induced preferentially in TLR5-competent hosts by the TLR itself and then drive malignant progression, whereas IL-17 may increase malignant progression only in the absence of both IL-6 and functional TLR5 signaling. Yet, both IL-6 and IL-17 are affected by commensal microbiota and eventually drive systemic changes that impact the immune contexture and foster tumor progression.

That microbiota-TLR5 interactions in mice control systemic inflammation and cancer might also be relevant for breast and ovarian cancer patients (Figure 1). Tumors from breast cancer patients with $TLR5^{R392x}$ have higher

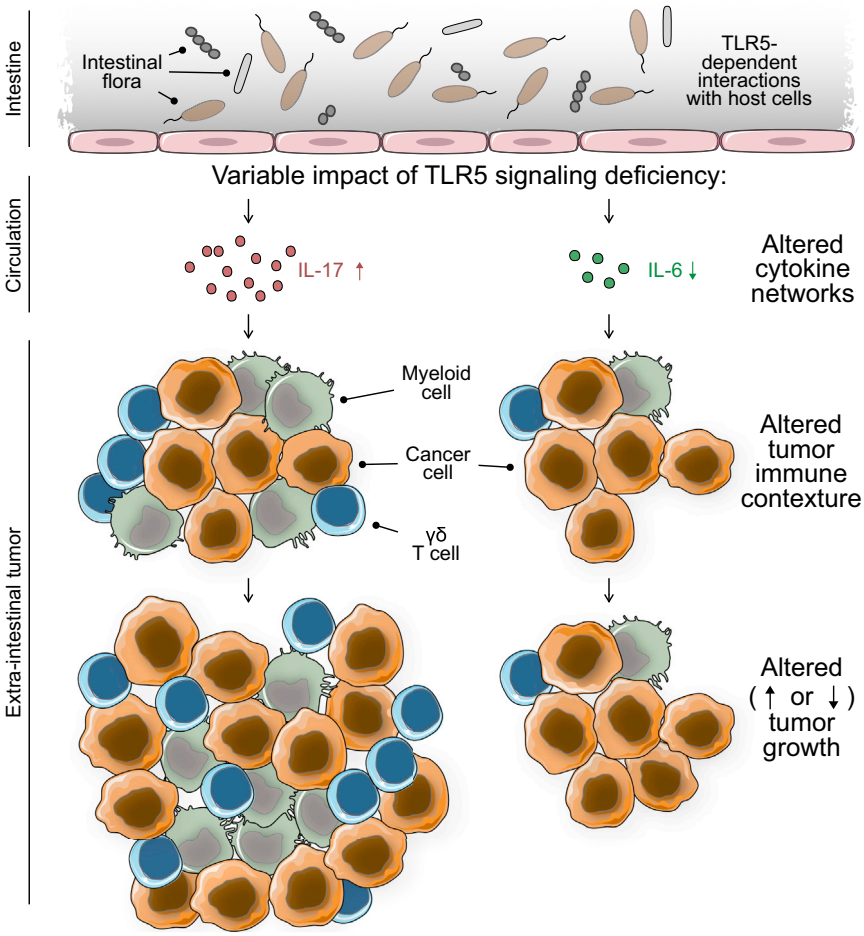

Figure 1. Host-Microbiota Interactions Impact Tumor Immune Contexture
Tumor growth is sensitive to the inflammatory milieu. Sensing of commensal bacteria residing in the intestines elicits systemic cytokine responses that can be altered by mutation of TLR5 or through oral antibiotics. Changes in systemic cytokine levels can either promote or impair tumor growth depending upon the type of cancer involved.

IL-17A transcript levels than those homozygous for the wild-type TLR5 allele. Retrospective analysis revealed that $TLR5^{R392x}$ breast cancer patients may have poorer overall survival compared to patients with wild-type TLR5 alleles. In contrast, retrospective analysis indicated that $TLR5^{R392X}$ ovarian cancer patients may have improved survival. This is consistent with mouse model data indicating that TLR5 signaling can accelerate ovarian cancer progression. As in the murine studies, TLR5-responsive ovarian cancer patients showed higher systemic IL-6 levels, whereas TLR5-unresponsive patients showed decreased numbers of γδ T cells and myeloid cells in the tumor stroma. All in all, ovarian cancer seems more IL-6 dependent in the presence of functional TLR5 signaling, whereas breast cancer may be preferentially driven by IL-17 in the absence of functional TLR5 (Figure 1). However, the relevance of, and dependence on, systemic IL-6 and IL-17 cytokines during tumor progression will require follow-up studies.

Interestingly, intestinal bacterial populations can regulate host immunity not only locally, but also systemically (Hooper et al., 2012; Ichinohe et al., 2011), but we do not yet know whether, or to what extent, certain intestinal microbial species define the immune contexture of, e.g., extraintestinal tumors. The present study indicates that the microbiome from *Tlr5*$^{-/-}$ mice cannot be transferred to co-housed wild-type mice, thereby suggesting that wild-type mice exhibit colonization resistance to the transferred bacteria. Instead, transplanting microbiota from *Tlr5*$^{-/-}$ mice to germ-free recipients may overcome colonization resistance barriers and facilitate mechanistic studies of specific microbes and their association with immune contexture and cancer development. Specific associations between microbiota and tumor immune contexture could provide a proxy for assaying the human tumor microenvironment by characterizing microbial species in the human intestines. The human tumor microenvironment is very difficult to characterize in vivo, so sequencing human microbiomes would be a convenient means to provide critical patient information.

Whether TLR5 loss-of-function can affect responses to anticancer therapy also remains unknown. Because the intestinal commensal microbiota can impact chemotherapy and immunotherapy (Viaud et al., 2013; Iida et al., 2013) and TLR5-deficiency perturbs the microbiota, the altered microbiota in a TLR5-deficient setting could also affect responses to treatment. Follow-up work examining how TLR5 signaling deficiency alters drug-induced immune responses and effective cancer control could prove valuable in personalizing cancer treatments. Because TLR5 is one of many innate immune regulators, evaluating common deficiencies or polymorphisms in other innate immune pathways could also provide insight into the mechanisms regulating systemic inflammation and influencing tumor growth and response to therapy.

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mTOR Signaling in Melanoma: Oncogene-Induced Pseudo-Senescence?

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Oncogene-induced senescence (OIS) is thought to be a barrier to malignant transformation resulting from the strong activation of oncogenes. In this issue of *Cancer Cell*, Damsky and colleagues suggest activation of mTORC1 and mTORC2 is required for OIS evasion in human melanomas harboring oncogenic *BRAF* mutations.

Cellular senescence was described more than five decades ago as an in vitro permanent growth arrest in primary fibroblasts after long-term culture. Subsequent work has shown that senescence can be induced by a wide variety of extra- and intracellular stresses. The molecular effectors of senescence have been well characterized, involving the activation of canonical tumor suppressors like p53, $p16^{Ink4a}$, and RB (Kuilman et al., 2010). Perhaps one of the most surprising findings regarding senescence has been the discovery that the strong activation of certain oncogenes, the very agents of cancer, will potently induce senescence in cells with intact p53 and $p16^{Ink4a}$-RB signaling (Serrano et al., 1997). The discovery of this "oncogene-induced senescence" (OIS) has troubled many cancer researchers. How could any normal cell ever give rise to a cancer if oncogene activation provokes a permanent growth arrest? A variety of explanations have been advanced in an attempt to resolve this seeming paradox. A few of the more popular notions have been that OIS (or even senescence in general) is merely an in vitro artifact, that p53 or $p16^{Ink4a}$ loss actually precedes strong oncogene activation in cancers, or that cellular senescence is in some cases not permanent as previously conceived.

Support for the occurrence of OIS in vivo is probably stronger for melanoma, a common and lethal human skin cancer, than for any other form of human cancer. The overexpression of common oncogenic drivers of melanoma, e.g., mutant Nras or Braf, potently induces melanocyte OIS in vitro in a $p16^{Ink4a}$- and p53-dependent manner. The expression of markers of senescence such as $p16^{Ink4a}$ can be demonstrated in precursor lesions of melanoma (nevi) harboring *BRAF* mutations, most commonly V600E. These observations have suggested that many nevi are would-be cancers but are successfully interdicted by the senescence machinery. The fact that the vast majority of nevi never progress to melanoma speaks to the robustness of OIS as a defense mechanism against cancer. It appears clearly true, however, that cells in a very small fraction of nevi evade OIS to cause the roughly 70,000 melanoma cases per year in the US alone.

Against this background, Damsky et al. (2015; in this issue of *Cancer Cell*) have tackled the OIS problem, focusing on melanoma. To do this, the authors use an array of state-of-the-art conditional murine models to comprehensively analyze the transitions from prearrested, to post-arrested, to transformed melanocytes. In accord with prior work (Dhomen et al., 2009; Michaloglou et al., 2005), the authors demonstrate that disruption of the *Cdkn2a* (*Ink4a/Arf*) locus alone is not sufficient to permit $Braf^{V600E}$-induced melanoma, suggesting that activated Raf signaling engages an antiproliferative response independent of Cdkn2a signaling (Figure 1). This observation is in accord with prior work suggesting that activated Ras-Raf signaling induces a compensatory repression of PI3K-Akt and Raf-Mek signaling (Courtois-Cox et al., 2006). In the present study, however, the combination of Braf activation and Cdkn2a loss was sufficient to prime progression of nevi to melanoma, presumably in the setting of additional stochastic events. By including a GFP expressed specifically in melanocytes in their murine models, the authors were able to purify uncultured