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REVIEWS

T cell exclusion, immune privilege, and the tumor microenvironment

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Effective immunotherapy promotes the killing of cancer cells by cytotoxic T cells. This requires not only that cancer-specific T cells be generated, but also that these T cells physically contact cancer cells. The coexistence in some patients of cancer cells and T cells that recognize them indicates that tumors may exhibit the phenomenon of immune privilege, in which immunogenic tissue is protected from immune attack. Here, we review the evidence that stromal cells of the tumor microenvironment mediate this restriction by excluding T cells from the vicinity of cancer cells. Overcoming this T cell checkpoint may thus enable optimal immunotherapy.

The microenvironment of tumors contains numerous cell types in addition to cancer cells, which include bone marrow–derived inflammatory cells, lymphocytes, blood vessels, fibroblastic cells, and the extracellular matrix composed of collagen and proteoglycans (*1*, *2*). The importance of a stromal microenvironment, especially one that has characteristics of a "wound" or regenerating tissue, has been recognized for at least a century (*3*), but its possible role in blunting an immune attack of cancer cells awaited the discovery of adaptive cellular immunity. In 1960, Klein and colleagues found that when mice developed primary methylcholanthrene-induced sarcomas, they also developed an antitumor immune response mediated by lymph node cells to a secondary challenge comprising cancer cells derived from the primary tumor (*4*). The paradoxical and critical finding of the study was that this anticancer immune response did not control the growth of the primary tumor, despite its ability to prevent the establishment of a secondary tumor comprising cancer cells derived from the primary tumor. In traditional immunological terminology, the primary tumor evaded immune control by establishing an immune-privileged microenvironment that is functionally analogous to that of certain normal tissues, such as the eye (*5*).

Unambiguous evidence for the inability in humans of a systemic immune response to eliminate immunogenic cancer cells was provided by Boon's studies 30 years later of the antigens that elicit specific $CD8^+$ T cell responses in melanoma patients (*6*). Cloned $CD8^+$ T cells from a melanoma patient were used to identify the antigen expressed by that patient's cancer: MAGE-A1. The explicit demonstration of the coexistence of a progressing melanoma with melanoma-specific T cells in this patient implicitly raised the question of why the T cells did not control the growth of the cancer. Immunoediting, or the elimination of immunogenic cancer cells (*7*), could be excluded, which left the possibility of immune suppression by the tumor microenvironment (TME). Despite this evidence that the presence of antigen-specific $CD8^+$ T cells alone may not be sufficient for the control of cancer, a major pharmaceutical company recently conducted phase III trials in patients with non–small cell lung cancer (NSCLC) of the clinical efficacy of vaccination with the MAGE-A3 antigen (MAGRIT, NCT00480025). The study did not meet its primary end point of extending disease-free survival and was discontinued in 2014. Moreover, Rosenberg and colleagues reported evidence of disease recurrence in melanoma patients despite very high levels of vaccine-induced circulating T cells and no evidence of antigen loss by the cancer cells (*8*).

The discovery of melanoma-specific T cells in patients led to another strategy to increase the frequency of cancer-specific T cells in patients, that of adoptively transferring large numbers of in vitro expanded tumor-infiltrating lymphocytes (TILs). As discussed elsewhere in this issue of *Science* (*9*), this approach has shown some efficacy, which has been of major importance to the field by serving as proof that the immune system has the potential to control cancer (*10*). However, adoptive T cell therapy (ACT) with TILs has not had the dramatic success of ACT with virus-specific $CD8^+$ T cells to immunodeficient bone marrow transplant recipients with cytomegalovirus infection (*11*) or Epstein-Barr virus–associated lymphoproliferative disorders (*12*). Differences in the microenvironments of virally infected tissues and cancers may account for these distinct outcomes, with the latter being immune-suppressive. Another important point of comparison is that the TME of solid cancers is likely to be fundamentally different to that of the leukemias, in which clinical trials of ACT with T cells expressing chimeric antigen receptors, so-called CAR T cells, have demonstrable efficacy (*9*). These findings raise the possibility that increasing the frequency of cancer-specific T cells, by whatever means, may be more effective if combined with an approach that alters the immune-suppressive TME.

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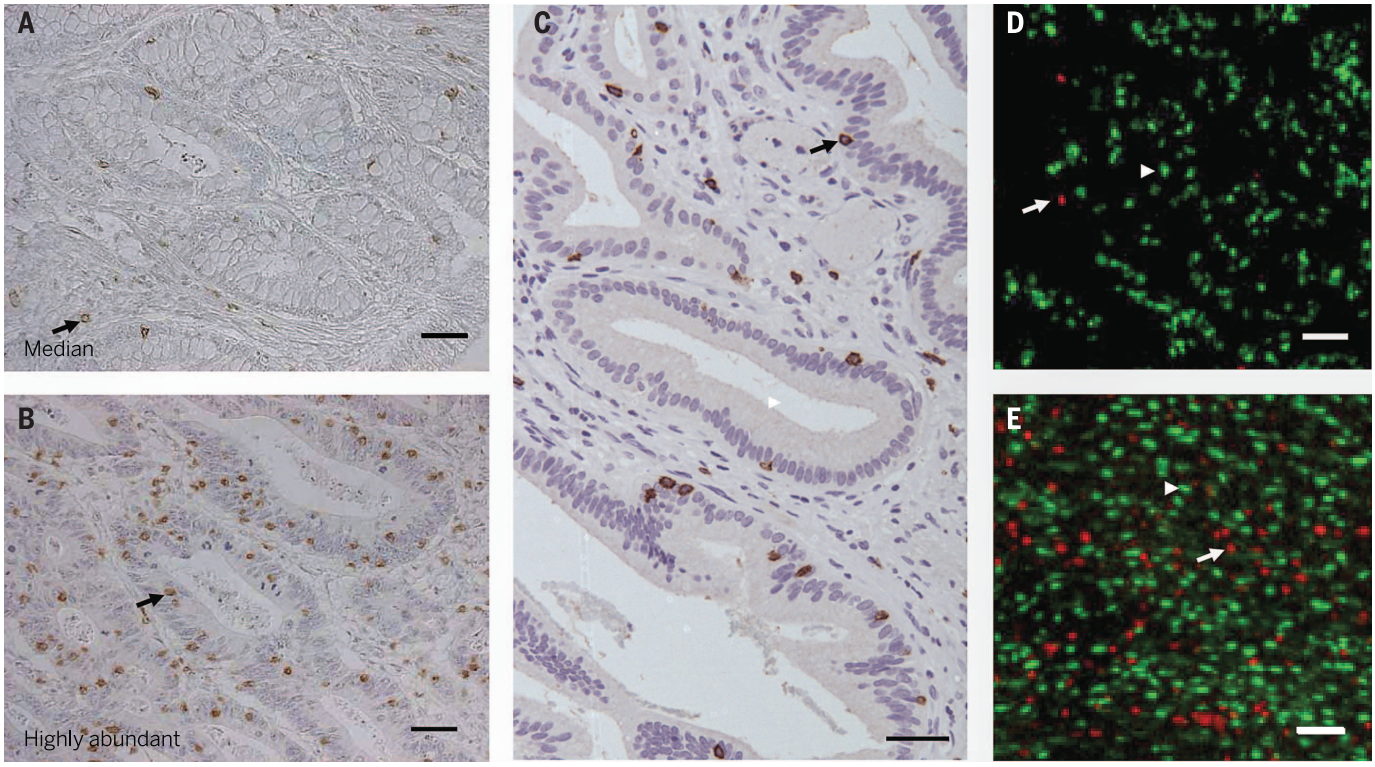

Fig. 1. Exclusion of T cells from human and mouse adenocarcinomas. (**A** to **C**) $CD3^+$ T cells are identified by immunoperoxidase stains of [(A) and (B)] human colorectal (*82*) and (C) human pancreatic ductal adenocarcinomas , demonstrating the presence of few [(A) and (C)] and many (B) intraductal T cells. (**D** and **E**) $CD3^+$ T cells and $p53^+$ cancer cells are identified by use of immunofluorescent stains of pancreatic ductal adenocarcinomas taken from (D) untreated mice and (E) mice that have been treated for 24 hours with the CXCR4 antagonist, AMD3100, demonstrating that T cell exclusion can be regulated by CXCR4 signaling (*29*). Scale bars, 50 μm. Arrows indicate examples of $CD3^+$ T cells, and arrowheads indicate examples of $p53^+$ cancer cells.

The more recent strategy of enhancing the function of effector T cells by targeting immunoregulatory membrane receptors has been successful in subsets of patients with melanoma, NSCLC, urothelial bladder cancer, and renal cell cancer (*13*–*18*). The therapeutic effect of blocking antibodies to the immune checkpoint regulators cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) and the programmed cell death protein 1 (PD-1)/PD-L1 receptor-ligand pair is covered in detail elsewhere in this issue of *Science* (*19*), and we briefly discuss them here because these therapies relate to the TME. For example, in the mice, anti-CTLA-4 therapy leads to clearance from the tumor of $Foxp3^+$ regulatory T cells (T_{reg} cells) (*20*), which may impair the functions of effector T cells at that site (*21*). Cancer cells—as well as infiltrating monocytic cells, including dendritic cells (DCs) and macrophages—express PD-L1 (*16*, *17*, *22*, *23*), which suppresses the proliferative and effector responses of T cells by engaging the inhibitory PD-1 receptor on these cells. Nevertheless, it has become apparent that even if these T cell checkpoint antagonists overcome some of the immune-suppressive effects of the TME, there may be other, more fundamental inhibitory reactions in the TME to explain why most patients—especially those with microsatellite stable colorectal cancer (CRC), ovarian cancer, prostate cancer, and pancreatic ductal adenocarcinoma (PDA)—rarely exhibit objective responses to these therapies (*14*, *15*, *24*).

A clue to the nature of this dominant immune suppression mediated by the TME comes from studies that have examined the spatial relationship of $CD8^+$ effector T cells to cancer cells in three of the tumors that did not respond to anti-PD-1/anti-PD-L1: CRC, ovarian cancer, and PDA (Fig. 1). In 1998, the exclusion of $CD8^+$ T cells from the vicinity of cancer cells in CRC was shown to correlate with a poor long-term clinical outcome (*25*), an observation that was confirmed and extended by Galon and colleagues in 2006 (*26*). Exclusion of T cells from the vicinity of cancer cells was also found in ovarian cancer (*27*, *28*) and PDA (*29*). Thus, the tumor immunology field provided evidence more than 10 years ago that the TME can limit the capacity of T cells to accumulate among cancer cells. It is reasonable to conclude that until this problem is circumvented, the full potential of other approaches to T cell–mediated tumor immunotherapy, such as augmenting the numbers and function of cancer-specific T cells, may not be realized.

Fortunately, studies over the past several years have begun to explain how this form of immune suppression is mediated. Preclinical studies in mouse models of cancer now implicate the major stromal cell types of the TME, cancer-associated fibroblasts (CAFs) and myelomonocytic cells, including several subsets of cells within the general designation of myeloid-derived suppressor

Table 1. Myelomonocytic cells and CAFs control the accumulation of T cells.

TUMOR	TARGET	CELL TYPE AFFECTED BY THERAPEUTIC INTERVENTION	REFERENCE
B16 melanoma-GM-CSF	CCR2	Monocytes	(*30*)
PDA	GM-CSF	MDSCs	(*31*)
PDA	GM-CSF	MDSCs	(*32*)
Cervical, Breast	CSF-1R	Monocytes, TAMs	(*33*, *36*)
PDA	CXCR4	Likely T cells (CXCL12 is produced by CAFs)	(*29*)
PDA	CSF-1R	Monocytes, TAMs	(*34*)
Prostate	CSF-1R	Monocytes, TAMs	(*35*)

cells (MDSCs) and tumor-associated macrophages (TAMs), as being responsible for restricting the accumulation of T cells in the vicinity of cancer cells (*29*–*36*) (Table 1). As would be predicted, overcoming this restriction revealed the antitumor effects of a T cell checkpoint antagonist that had been ineffective when administered as monotherapy. Moreover, as will be discussed, the tumor vasculature also plays an active role in restricting T cell entry into the TME. Fortunately, for each immune suppressive element of the TME there are therapeutic entities that are potentially suitable for administration to patients.

Control by the TME of the extravasation of T cells from the circulatory system into tumors

After the priming of cancer-specific T cells in the lymph nodes that drain the tumor, these T cells traffic via the circulatory system to the tumor. Studies have shown that the TME may regulate the accumulation of T cells in tumors at the initial step of their interaction with local blood vessels. Given that many other immune cells that compose the TME are nonetheless able to extravasate from the circulation (*1*), there must be means by which these distinct cell types are differentially recruited into the tumor. One mechanism for cellular discrimination comes from the release of chemokines that preferentially recruit certain immune cell types over others. Another is the capacity of the TME to posttranslationally alter chemokines. For example, the production of reactive nitrogen species by MDSCs within the TME induces nitration of CCL2 (N-CCL2), which

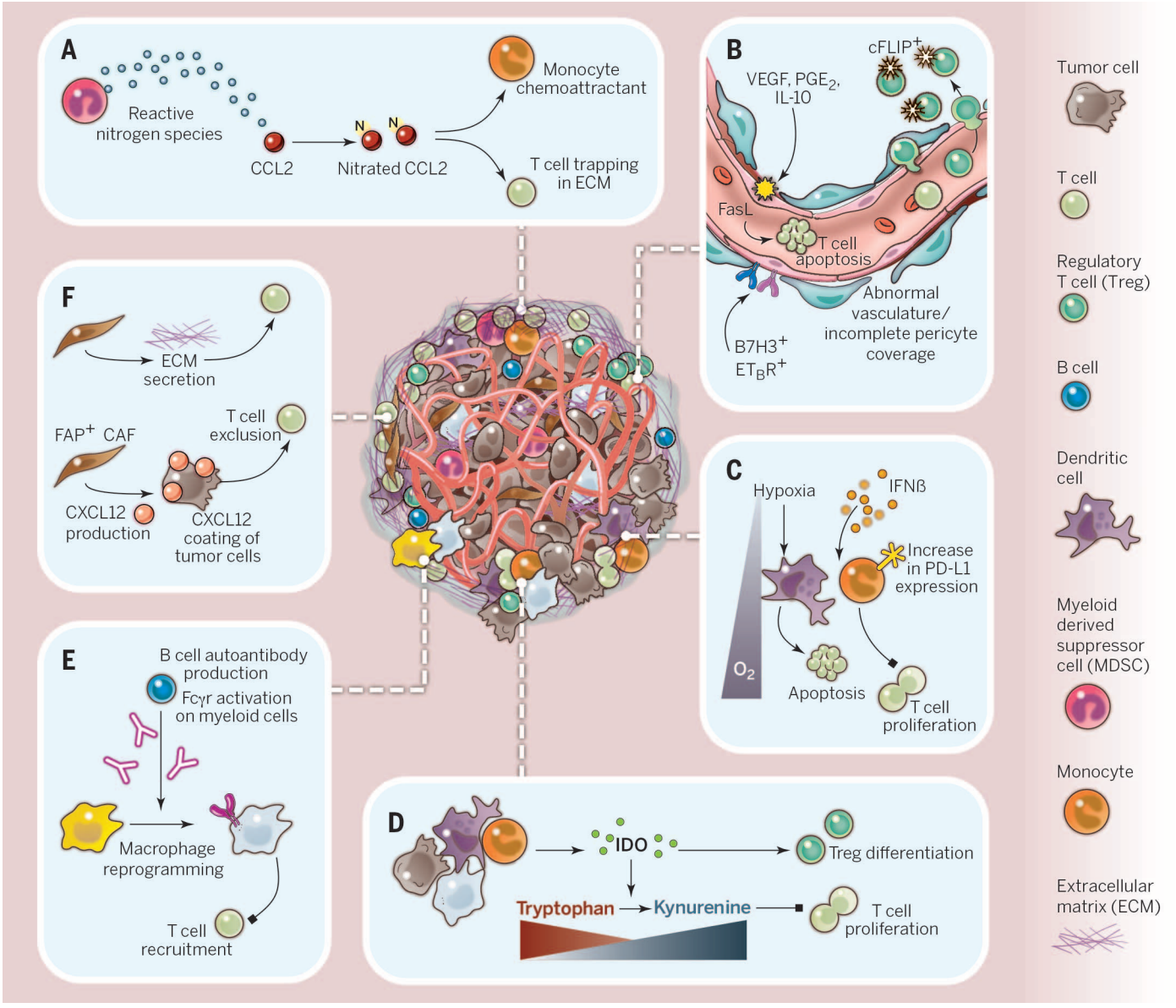

Fig. 2. Mechanisms of TME-driven immune suppression. A plethora of noncancerous cells in the TME regulate the infiltration, accumulation, and proliferation of T cells in tumors, with representative examples shown here. (**A**) T cell recruitment can be blocked by nitration of the chemokine CCL2, resulting in T cell trapping in the stroma. (**B**) The tumor vasculature plays a complex role in preferential recruitment of other immune cells over T cells, in part through endothelial cell (EC)–specific expression of FasL, ET_BR, and B7H3. (**C**) PD-L1 expression can be up-regulated in myelomonocytic cells, in addition to tumor cells, and is driven in part by hypoxic conditions in the TME and the production of cytokines, such as IFNβ. (**D**) The aberrant production of metabolites in the TME, such as the pathway regulated by IDO, can result in a multitude of effects directly on T cell functions and indirectly via other cells such as T_{reg} cells. (**E**) B cells can regulate the phenotype of TAMs resulting in suppression of CD8 cells. (**F**) Cancer-associated fibroblasts (CAFs) have multiple functions in the TME, in part through extracellular matrix (ECM)–mediated T cell trapping and CXCL12-regulated T cell exclusion.

results in the trapping of T cells in the stroma that surrounds tumor cells of human colon and prostate cancers (Fig. 2A) (*37*). In contrast, N-CCL2 still attracts monocytes, potentially contributing to the differential recruitment of these distinct immune cell types in vivo. Inhibitors of CCL2 nitration enhanced the accumulation of TILs in the corresponding animal models and resulted in improved efficacy of ACT.

Even if the appropriate chemotactic signals for the extravasation and recruitment to the tumor of T cells are present, the vasculature can override their effects and actively exclude T cells (Fig. 2B), a function that may distinguish between the effector T cells and other leukocyte populations, such as T_{reg} cells and myeloid cells. Insights into the mechanism of how this might occur have come from studies comparing T cell–rich and T cell–poor tumors. These studies revealed that the apoptosis inducer Fas ligand (FasL) is expressed in the tumor vasculature of multiple tumor types, including ovarian, colon, prostate, breast, bladder, and renal cancer (*38*). In tumors with high levels of endothelial FasL, there are few $CD8^+$ T cells but abundant T_{reg} cells, which may be protected against FasL-mediated killing by their relatively high expression of the apoptosis inhibitor, c-FLIP. Accordingly, in preclinical models FasL inhibition resulted in a substantial increase in the influx of tumor-rejecting T cells relative to T_{reg} cells, which led to T cell–dependent tumor suppression. FasL expression itself is induced by the TME-derived immunosuppressive factors vascular endothelial growth factor (VEGF), prostaglandin E_2 (PGE_2), and interleukin-10 (IL-10), suggesting that multiple

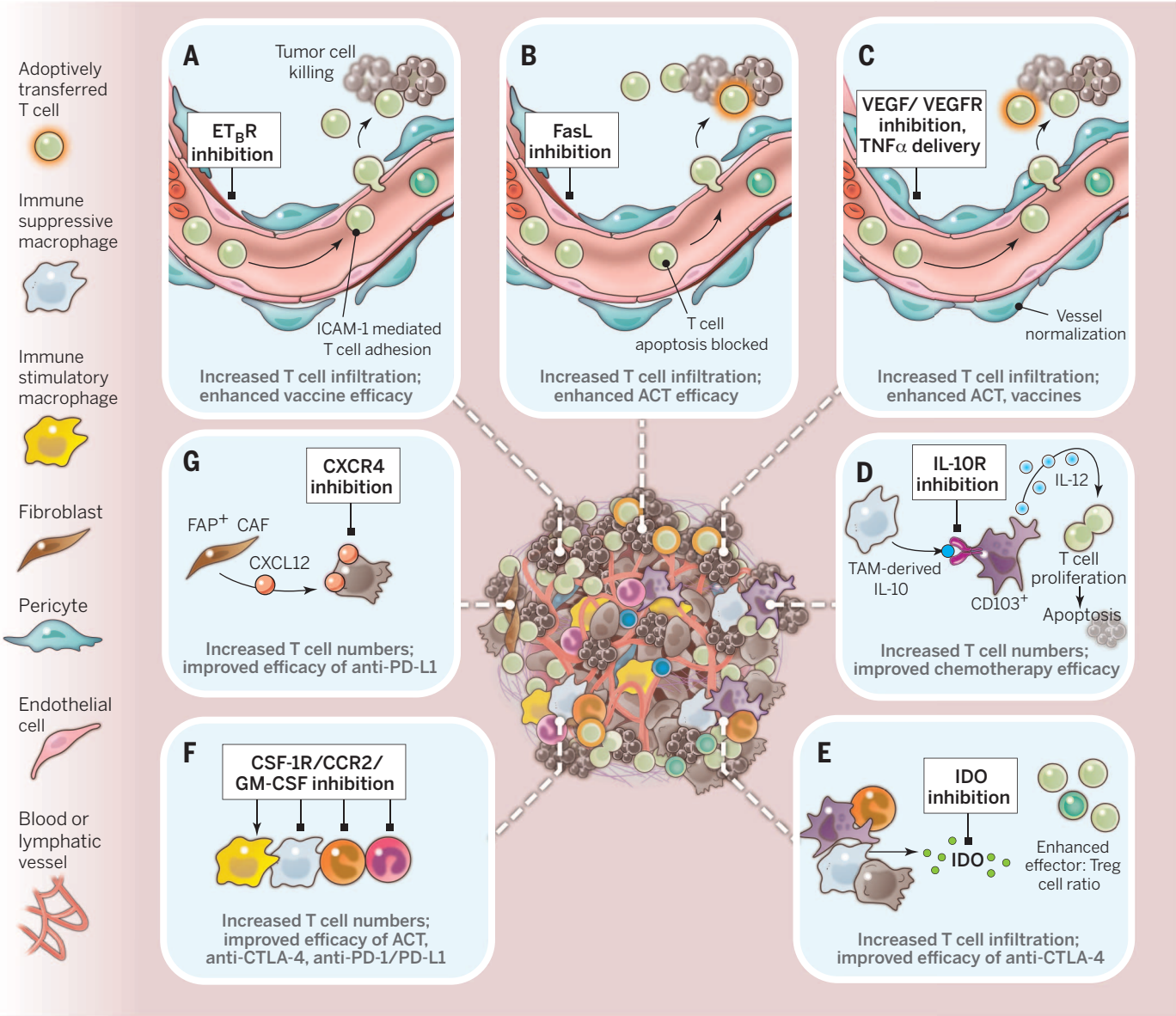

Fig. 3. Therapeutic strategies to overcome immune suppression in the TME. A number of vascular-targeted therapies result in increased T cell infiltration and improved efficacy of different immunotherapies such as adoptive cell therapy and anticancer vaccines. These include (**A**) ET_BR inhibition, (**B**) FasL inhibition, and (**C**) VEGF/VEGFR/TNFα inhibition. (**D**) Dendritic cells (DCs) can have opposing functions in the TME, either supporting or suppressing tumor development. $CD103^+$ DCs have an immune stimulatory function, resulting in IL-12 secretion and T cell replication when the immune-suppressive cytokine receptor IL-10R is inhibited. (**E**) IDO inhibition has multiple effects on TILs, including augmenting T cell expansion and preventing their differentiation into T_{reg} cells. (**F**) Various myelomonocytic cells suppress T cell numbers and/or functions; this suppression can be relieved by inhibition of a number of cytokine signaling pathways indicated here, resulting in depletion or reeducation of these cells in the TME. Further information is provided in Table 1. (**G**) Inhibition of CXCL12/ CXCR4 downstream of FAP^+ CAFs in the TME leads to T cell accumulation and increased efficacy of anti-PD-L1 therapy.

networks of cellular interactions may converge to establish immune tolerance. In ovarian cancer elevated VEGF levels, and expression of the immune regulatory ligand B7H3 (CD276), or the endothelin B receptor (ET_BR) on tumor vessels correlates with decreased T cell infiltration and worse clinical outcome (*27*, *39*, *40*). Pharmacological inhibition of ET_BR increased T cell adhesion to endothelial cells in an intercellular adhesion molecule-1 (ICAM-1)–dependent manner, resulting in significantly enhanced TIL numbers in mice and a corresponding tumor response to an otherwise ineffective anticancer vaccine (Fig. 3A) (*40*). Similarly, FasL inhibition also improves the efficacy of ACT (Fig. 3B) (*38*). The improved efficacy of these distinct TME-directed immunotherapies was not as a consequence of a more effective systemic antitumor immune response but could be attributed to increased effector T cell infiltration into tumors.

Attention has also been focused on anti-angiogenic therapies as a potential means to enhance the efficacy of immunotherapy (*41*). Anti-angiogenic inhibitors targeting VEGF and its receptor VEGFR2, which are approved for clinical use in multiple cancers (*42*), induce vascular normalization. This, in turn, increases TILs and improves the efficacy of ACT and cancer vaccines in preclinical models (Fig. 3C) (*43*, *44*). In relation to the next section of this Review, VEGF impairs the maturation of DCs (*45*), so that anti-VEGF therapy has an additional means by which it could enhance intratumoral immune responses. Further support for the importance of vascular normalization has come from the finding that deleting the regulator of G-protein signaling, Rgs5 (*46*), reduced vessel leakiness and hypoxia, enhanced T cell infiltration into mouse pancreatic neuroendocrine tumors, and prolonged animal survival. Therefore, from an immunotherapeutic perspective, vascular normalization is likely to be more efficacious than anti-angiogenic therapies that result in vessel destruction, as exemplified by the differential effects of delivering the pro-inflammatory cytokines interferon-γ (IFN-γ) versus tumor necrosis factor-α (TNF-α). Only targeted delivery of the latter, which was reported to normalize tumor blood vessels and increase $CD8^+$ T cell infiltration, enhanced vaccine and ACT therapies (Fig. 3C) (*47*, *48*).

TME-mediated regulation of the local replication of T cells within tumors

The extravasation of cancer-specific T cells into the tumor is a necessary, but not sufficient, step in the immune control of cancer. For effective immune killing of cancer cells, these T cells must also locally replicate to further increase their frequency, avoid being killed themselves by hostile elements of the TME, and overcome barriers that restrict their distribution to the stroma and away from cancer cells. The TME affects all three of these intratumoral T cell responses.

Although the site of the self-renewing T cells that are clonally expanding in response to cancer cell–associated antigens is likely to be the draining lymph nodes, the enrichment of cancer-specific effector T cells within the tumor relative to their frequency in the periphery indicates that replication of effector T cells within the tumor also occurs. Findings in preclinical models suggest that the TME may be the major site of clonal expansion of cancer-specific T cells (*49*, *50*), and that the $CD8^+$ T cell replicative response at this site is orchestrated by the $CD103^+$, Baft3-dependent DC, which can efficiently cross-present cancer cell antigens (*51*, *52*). The dependence of T cell–mediated tumor regression on the intratumoral presence of $CD103^+$ DCs suggests that therapeutic interventions that enhance their numbers or capacity for driving T cell replication in the TME may contribute to tumor control. Among such strategies are antibodies to the IL-10R, which in a mouse model of mammary carcinoma neutralized the effects of IL-10 produced by TAMs, relieved the suppression of IL-12 production by intratumoral DCs, and improved the $CD8^+$ T cell–dependent antitumor effects of chemotherapy (Fig. 3D) (*53*). A similar outcome was achieved by neutralizing CSF-1, which impaired the intratumoral accumulation of TAMs (*32*, *33*). Yet another strategy is the administration of antibody-IFN-β complexes, targeted against oncogenic receptors, such as EGFR, that activate intratumoral DCs for cross-presentation of antigen to $CD8^+$ T cells (*54*). Tumor eradication resulted when PD-L1, which also was induced by IFN-β acting on DCs, was neutralized, demonstrating the recurring theme in the immune system that activating stimuli prompt compensatory inhibitory responses. DC function also may be adversely affected by the hypoxic conditions characteristic of the TME, which induces PD-L1 expression on DCs and other myelomonocytic cells (Fig. 2C) as a result of HIF-1α binding directly to a hypoxia-responsive element in the PD-L1 promoter (*55*). Even the aerobic glycolysis of cancer cells may antagonize local immune reactions via its increased production of lactate, which induces the M2 polarization of TAMs (*56*). An M1 to M2 phenotypic transition of intratumoral macrophages has also been reported after the induction of cancer cell apoptosis in human and mouse gastrointestinal stromal tumors by the administration of the KIT oncoprotein inhibitor, imatinib (*57*). It should be noted that the designation of M1 and M2 polarization states undoubtedly represent an oversimplification of the complexity of macrophage biology (*58*) and that at least six different TAM subpopulations have been reported (*59*). Therefore, descriptors of TAM phenotypes in the TME are likely to be most informative in investigating and therapeutically targeting these cells.

In addition to altering T cell replication indirectly via effects on myeloid cells, the TME may directly impair intratumoral T cell proliferation. Indole 2,3-dioxygenase (IDO)—which can be expressed by DCs, MDSCs, and cancer cells—catabolizes tryptophan and generates kynurenine (Fig. 2D). Both the deprivation of tryptophan and the generation of its metabolic product inhibit clonal expansion (*60*, *61*). IDO also promotes the conversion of naïve T cells to T_{reg} cells and increases IL-6 expression, which augments MDSC functions (*62*). Accordingly, IDO1 genetic deficiency is associated with reduced tumor burden and metastasis and enhanced survival in mouse models of lung and breast cancer (*62*). The therapeutic potential of inhibiting IDO, in combination with the T cell checkpoint antagonist anti-CTLA-4, has been demonstrated in the B16 melanoma model and was associated with increased accumulation of intratumoral T cells (Fig. 3E) (*63*). Last, the capacity of IDO to block the reprogramming of T_{reg} cells to helperlike cells by suppressing the loss of the transcription factor Eos, and the corresponding transcriptional program it regulates, exemplifies another means by which this enzyme promotes immune suppression within the TME (*64*).

Control by the TME of the viability of T cells within tumors

The TME can also limit the viability of T cells. Both IDO and PD-L1 not only may impair the intratumoral proliferation of effector T cells but may also induce apoptosis of these cells. Products of myelomonocytic cells that cause the apoptosis of T cells include FasL, TNF-α, and TNF-related apoptosis inducing ligand (TRAIL). In addition to these known effectors of death, previously unidentified pathways that control the viability of intratumoral T cells may be discovered by innovative, unbiased approaches. For example, an in vivo, pooled short hairpin RNA screen identified Ppp2r2d as a key regulator promoting T cell apoptosis and suppressing T cell proliferation within the TME (*65*).

Interventions that target intratumoral TAMs and MDSCs can also lead to reduced tumor burdens in preclinical models, in both T cell–dependent and T cell–independent ways. For instance, inhibiting chemokine receptor type 2 (CCR2) (*30*), colony-stimulating factor-1 receptor (CSF-1R) (*33*, *34*, *36*), and granulocyte macrophage colony-stimulating factor (GM-CSF) (*31*, *32*) in preclinical models of melanoma, pancreatic, breast, and prostatic carcinoma increased intratumoral T cells and controlled tumor growth, especially when combined with anti-CTLA-4 or anti-PD-1/PD-L1 (Table 1 and Fig. 3F). Although these studies did not determine whether the increases in T cells were a consequence of enhanced viability or replication, they emphasize again how elements of the TME regulate the accumulation of effector T cells. Inhibition of CSF-1R in a preclinical model of proneural glioblastoma multiforme and in patient-derived glioma xenografts increased survival and caused regression of established tumors in an apparent T cell–independent manner that correlated with the reprogramming of macrophages away from an M2 phenotype (*66*). Similarly, an activator of TAMs, an agonistic antibody to CD40, when administered in combination with the chemotherapeutic drug gemcitabine, suppressed the growth of mouse PDA in a T cell–independent manner (*67*), suggesting that macrophages alone, when appropriately stimulated, may have potent anticancer functions. B cells have also been shown to regulate the phenotype of TAMs in the squamous cell carcinoma TME (Fig. 2E) (*68*). Correspondingly, B cell depletion reprogrammed TAMs, thus relieving their suppression of CD8 cells and enhancing chemotherapy efficacy. Another example

of how the antitumor effects of macrophages can be used therapeutically is an autochthonous mouse model of melanoma in which the melanoma-killing capability of these cells was revealed by depleting T_{reg} cells and neutralizing IL-10 (*69*). TAMs would also be the mediators of the antitumor effects of antibodies (*70*) and genetically engineered ligands (*71*) that interact with CD47 on cancer cells to prevent the CD47/signal regulatory protein–α (SIRPα) signaling system from suppressing the phagocytosis of antibody-coated cancer cells.

The TME regulates spatial distribution of T cells within tumors

Increased numbers of intratumoral, cancer-specific T cells will be of little import if T cells are restricted to the stroma and prevented from accumulating in the vicinity of cancer cells. CAFs, which may be identified by their expression of the membrane protein fibroblast activation protein-α (FAP), have been shown to have two means by which they can mediate this restriction, the first of which is a physical exclusion mediated by the extracellular matrix that they produce (Fig. 2F). Live cell imaging of lung tumor tissue slices from patients revealed active T cell motility in regions of loose fibronectin and collagen, whereas T cells migrated poorly in dense matrix areas surrounding tumor nests (*72*). When either collagenase was added to reduce matrix rigidity, or the chemokine CCL5 was experimentally produced by tumor cells, there was increased T cell movement out of the stromal regions and into contact with cancer cells.

The second means by which FAP^+ CAFs exclude T cells involves their biosynthesis of CXCL12 (Fig. 2F). Conditionally depleting these cells from the stroma of an ectopic, transplanted tumor (*73*) and of an autochthonous PDA (*29*) allowed preexisting cancer-specific T cells to rapidly control tumor growth and revealed the antitumor effects of anti-PD-L1. However, depleting FAP^+ stromal cells is not a reasonable therapeutic option unless the depletion can be limited to the TME because these cells carry out essential functions in several normal tissues (*74*). The recent report of "reprogramming" these cells in the TME by administration of a vitamin D analog (*75*) may be one means of circumventing this problem. Another may be to block their immune suppressive mechanism. In a preclinical mouse model of PDA, FAP^+ CAFs produce the chemokine CXCL12, which is bound by the PDA cancer cells, which had been previously reported for cancer cells in human PDA, CRC, and ovarian cancer (*76*–*78*). Because FAP^+ stromal cells also accumulate in nontransformed, inflammatory lesions, this "coating" of cancer cells may reflect a means by which "injured" epithelial cells protect themselves from adaptive immune attack. Administering an inhibitor of CXCR4, the receptor for CXCL12, to the PDA-bearing mice caused the rapid accumulation of T cells among cancer cells, arrest of tumor growth, and tumor sensitivity to anti-PD-L1 (Fig. 3G) (*29*). How the cancer cell–bound CXCL12 excludes T cells has not yet been shown, although the mechanism must involve either T cells or myelomonocytic cells because they, and not cancer cells or FAP^+ CAFs, express CXCR4 in this model.

Conceptual challenges and therapeutic opportunities

Among the challenges that remain for understanding the immune suppressive roles of the TME, three are foremost: comprehending the mechanisms by which the TME excludes T cells, determining whether the TME of primary and metastatic tumor sites differ, and assessing the potential clinical efficacy of interventions that affect the TME. The preclinical studies in mice that showed that inhibiting CCR2, CSF-1/CSF-1R, GM-CSF, or CXCR4 improved immune control of tumor growth also showed that these interventions shared a capacity for increasing the frequency of T cells among cancer cells (Fig. 3). Because targeting CCR2 and CSF-1/CSF-1R diminishes the accumulation of CCR2-expressing cell types, including bone marrow–derived TAMs and DCs, one must conclude that at least one function of these cells is to suppress the accumulation of intratumoral T cells. However, given that these cells are distributed in both the stromal and cancer cell regions of tumors, it is not readily apparent how they can selectively exclude T cells only from the vicinity of the cancer cells. On the other hand, the distribution of intratumoral CXCL12, which is associated with cancer cells, does correlate, albeit inversely, with that of T cells, so that the hypothesis that CXCL12 is involved with T cell exclusion would be reasonable and is supported by the antitumor outcome of inhibiting CXCR4. Even here a mechanism that may account for this effect of CXCR4, other than T cell "repulsion" (*79*), is not apparent. For the moment, then, one may only suggest that because CSF1R- and CCR2-dependent cells and CXCR4 signaling are both required for the exclusion of T cells, they are elements of a single pathway that mediates this dominant immune suppressive process.

Regarding the TME of metastatic sites, most preclinical and clinical analyses to date have been restricted to primary tumors. It has been noted earlier that mice in which an immune response has been induced by growth of a primary methycholanthrene-induced sarcoma prevent the establishment of a secondary tumor by these sarcoma cells (*4*). In a preclinical model of spontaneous melanoma, cancer cells were found to disseminate early but to remain in a dormant state that was mediated, at least in part, by $CD8^+$ T cells (*81*). Consistent with this report of immune-induced metastatic dormancy is a study that found metastases in another mouse model that grew rapidly in association with the exclusion of $CD8^+$ T cells (*81*). A challenge will be to determine whether the immune-suppressive intensity of the TMEs of metastatic lesions may vary, with dormant metastases being dominated by immune control and growing lesions exhibiting immune suppression.

With respect to clinically assessing the effects of altering the TME for the purpose of increasing the frequency of intratumoral effector T cells, the academic oncologist already has several agents available that are specific for the same targets in humans that have regulated this process in mouse cancers: IDO inhibitors, CSF-1R inhibitors, CCR2-specific antibodies, and an inhibitor of CXCR4. Examples of each are already in clinical trials in human cancer patients, usually as monotherapies. There is an obvious rationale to combine those agents that are found to augment the intratumoral accumulation of effector T cells with therapies that improve the response of T cells to TCR ligation, such as antibodies to PD-1 and PD-L1, or increase the overall frequency of cancer-specific T cells, such as vaccines and ACT.

Last, recognition of the function of the TME in excluding T cells prompts an interest in the identity of the normal biological circumstance that is responsible for the development of this phenomenon. Tumor immunologists currently consider mutated genes to be the major source of antigens in cancer cells that T cells respond to, but some cancers that have a low mutational burden may elicit cancer-specific $CD8^+$ T cells, as exemplified by the mouse model of PDA (*29*). Is it possible that nontransformed epithelial cells in regenerating tissues also express immunogenic neoantigens, a circumstance that would select for an immune suppressive microenvironment? The frequent occurrence of the immune suppressive elements of the TME, myelomonocytic cells, and FAP^+ stromal fibroblasts in regenerating tissues is consistent with this conjecture and merits further investigation.

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REVIEWS

Cancer and the microbiota

Wendy S. Garrett[1,2,3,4]

A host's microbiota may increase, diminish, or have no effect at all on cancer susceptibility. Assigning causal roles in cancer to specific microbes and microbiotas, unraveling host-microbiota interactions with environmental factors in carcinogenesis, and exploiting such knowledge for cancer diagnosis and treatment are areas of intensive interest. This Review considers how microbes and the microbiota may amplify or mitigate carcinogenesis, responsiveness to cancer therapeutics, and cancer-associated complications.

The relationship between cancer and microbes is complex. Although cancer is generally considered to be a disease of host genetics and environmental factors, microorganisms are implicated in ~20% of human malignancies (*1*). Microbes present at mucosal sites can become part of the tumor microenvironment of aerodigestive tract malignancies, and intratumoral microbes can affect cancer growth and spread in many ways (*2*–*6*). In counterpoise, the gut microbiota also functions in detoxification of dietary components, reducing inflammation, and maintaining a balance in host cell growth and proliferation. The possibility of microbe-based cancer therapeutics has attracted interest for more than 100 years, from Coley's toxins (one of the earliest forms of cancer bacteriotherapy) to the current era of synthetic biology's designer microbes and microbiota transplants. Thus, interrogation of the roles of microbes and the microbiota in cancer requires a holistic perspective.

The ways in which microbes and the microbiota contribute to carcinogenesis, whether by enhancing or diminishing a host's risk, fall into three broad categories: (i) altering the balance of host cell proliferation and death, (ii) guiding immune system function, and (iii) influencing metabolism of host-produced factors, ingested foodstuffs, and pharmaceuticals (Fig. 1). Assigning microbial communities, their members, and aggregate biomolecular activities into these categories will require a substantial research commitment. This Review discusses how microbes and the microbiota may contribute to cancer development and progression, responsiveness to cancer therapeutics, and cancer-associated complications.

Microbial contributions to carcinogenesis

Of the estimated 3.7×10^{30} microbes living on Earth (*7*), only 10 are designated by the International Agency for Cancer Research (IACR) as carcinogenic to humans (*1*). Although most of these carcinogenic microbes colonize large percentages of the human population, only a subset of affected individuals develop cancer, because host and microbial genotypes influence cancer susceptibility.

Tumors arising at boundary surfaces, such as the skin, oropharynx, and respiratory, digestive, and urogenital tracts, harbor a microbiota, which complicates cancer-microbe causality. Enrichment of a microbe at a tumor site does not connote that a microbe is directly associated, let alone causal, in disease. Rather, microbes may find a tumor's oxygen tension or carbon sources permissive and take advantage of an underused nutritional niche. Decreased abundances of specific microbes may also place a host at enhanced risk for cancer development at sites local or distant from this microbial shift. Thus, rigorous frameworks for interpreting tumor-associated microbiota data are essential (*2*).

Oncomicrobes, shifting the balance of when to die and when to grow

Bona fide oncomicrobes—microbes that trigger transformation events in host cells—are rare. Beyond the 10 IACR-designated microbes, there are a handful of other microorganisms with robust but fewer aggregate data supporting their role in human carcinogenesis. As many of these and their carcinogenic mechanisms have been recently reviewed (*2*–*6*, *8*), select activities representing common pathways by which microbes influence cancer will be highlighted.

Human oncoviruses can drive carcinogenesis by integrating oncogenes into host genomes. Human papillomaviruses (HPV) express oncoproteins such as E6 and E7. Data from recent genomic analyses of HPV^+ cervical cancers suggest that viral integration also selectively triggers amplification of host genes in pathways with established roles in cancer (*9*).

Microbes also drive transformation by affecting genomic stability, resistance to cell death, and proliferative signaling. Many bacteria have evolved mechanisms to damage DNA, so as to kill competitors and survive in the microbial world. Unfortunately, these bacterial defensive factors can lead to mutational events that contribute to carcinogenesis (Fig. 2). Examples include colibactin encoded by the *pks* locus [expressed by B2

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