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REVIEW

Modulation of the immune microenvironment by tumor-intrinsic oncogenic signaling

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The development of cancer immunotherapies has been guided by advances in our understanding of the dynamics between tumor cells and immune populations. An emerging consensus is that immune control of tumors is mediated by cytotoxic CD8⁺ T cells, which directly recognize and kill tumor cells. The critical role of T cells in tumor control has been underscored by preclinical and clinical studies that observed that T cell presence is positively correlated with patient response to checkpoint blockade therapy. However, the vast majority of patients do not respond or develop resistance, frequently associated with exclusion of T cells from the tumor microenvironment. This review focuses on tumor cell–intrinsic alterations that blunt productive anti-tumor immune responses by directly or indirectly excluding effector CD8⁺ T cells from the tumor microenvironment. A comprehensive understanding of the interplay between tumors and the immune response holds the promise for increasing the response to current immunotherapies via the development of rational novel combination treatments.

Introduction

Cancer immunotherapy has revolutionized the landscape of cancer therapies over the past decade. While long-term survival is observed for a fraction of cancer patients, the majority of patients currently do not benefit from immunotherapy treatments (Pardoll, 2012; Topalian et al., 2015; Ribas and Wolchok, 2018). Pre-clinical and clinical models indicate that the presence of tumor-reactive cytotoxic CD8⁺ T cells is required for the response to checkpoint blockade therapy, the most prevalently used immunotherapy (Ji et al., 2012; Taube et al., 2012; Tumeh et al., 2014; Van Allen et al., 2015; Chen et al., 2016). Checkpoint blockade therapy targets inhibitory "checkpoints" such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or programmed cell death 1 (PD-1), expressed on dysfunctional effector T cells, or ligands such as programmed death-ligand 1 (PD-L1) expressed on tumor or stromal cells. Monoclonal antibodies targeting those cell surface molecules disrupt inhibitory interactions, allowing the reinvigoration of an effector T cell response (Ribas and Wolchok, 2018; Hui, 2019). The observation of a positive correlation between CD8⁺ T cell presence and response to checkpoint blockade therapy has led to the adoption of T cell presence or the presence of a T cell gene signature as a de facto biomarker for a response to checkpoint blockade therapy. Tumors with a T cell–inflamed tumor microenvironment (TME) are often referred to as "hot" tumors. Conversely, tumors lacking T cell infiltration, often referred to as immunological deserts or "cold" tumors, are typically not responsive to checkpoint blockade therapy. While immune infiltration into the tumor, predominantly by myeloid cell types, which include macrophages and myeloid-derived suppressor cells (MDSC), has been reported to enhance tumorigenesis (Hanahan and Weinberg, 2011; Kumar et al., 2016; DeNardo and Ruffell, 2019), this review focuses on tumor-immune interactions affecting the infiltration of tumor-reactive T cells.

The key steps and features of an anti-tumor immune response are referred to as the cancer-immunity cycle (Chen and Mellman, 2013). The process is initiated when the tumor cells produce danger signals sensed predominantly by dendritic cells (DC) and other cells of the antigen presenting cell (APC) compartment. These APC acquire tumor-derived peptides (antigens) and, following activation, migrate into peripheral lymphoid organs to activate naive T cells specific for tumor-derived antigens. Activated T cells then traffic or home to the tumor site, where they exert their effector functions on the tumor cells. Cytotoxic CD8⁺ T cells are indispensable in the cancer-immunity cycle as they directly recognize and kill tumor cells (Martínez-Lostao et al., 2015). The major steps in the cancer-immunity cycle can be referred to as sensing, priming, homing, and killing (Fig. 1).

Within the framework of the cancer-immunity cycle, non–T cell–inflamed tumors could arise due to disruption at each major step: sensing, priming, and homing. One hypothesis is

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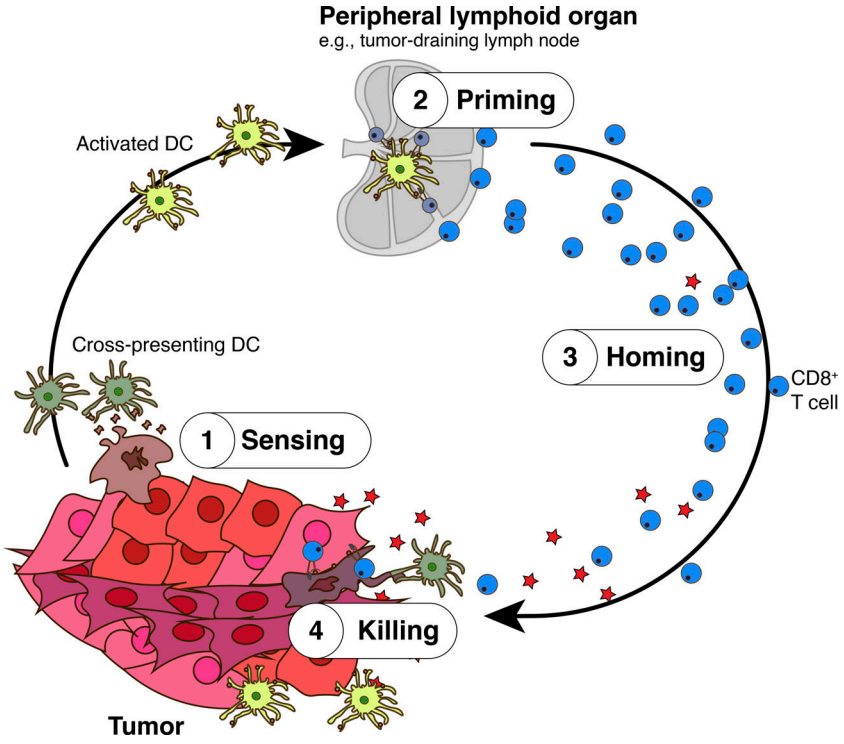

Figure 1. **A productive cancer-immunity cycle.** A productive anti-tumor immune response is first initiated when professional APCs (1) sense danger signals released by tumor cells and phagocytose tumor debris. This is largely accomplished by a particular subset of DC, the cross-presenting DC. These cells, now activated and loaded with tumor debris, can present tumor-derived peptides on MHCI directly to cytotoxic CD8⁺ T cells to (2) prime and activate the antigen-specific T cells. Activated T cells will (3) home to the tumor, following molecular cues, and will (4) kill tumor cells expressing the cognate peptide-MHCI. Dying tumor cells can continue to propagate the cycle. Such a response would result in a T cell–inflamed phenotype.

that tumors may simply not possess immunogenic antigens, so the cycle is halted at the priming stage. However, in analyses across all solid cancers, mutational load measured by non-synonymous mutations as a proxy for neoantigen burden was not significantly different between T cell–inflamed and non–T cell–inflamed tumor samples. These data suggest that lack of neoantigens does not cause a non–T cell–inflamed TME (Spranger et al., 2016; Danilova et al., 2016). Instead, evidence is accumulating that tumor cell–intrinsic alterations in signaling pathways, previously described to drive tumorigenesis, can affect the cancer-immunity cycle by modulating the TME. Tumor cell–intrinsic alterations can mediate a non–T cell–inflamed phenotype by (1) excluding cells that contribute to a productive immune response (Fig. 2) and (2) attracting immunosuppressive populations actively excluding effector T cells (Fig. 3).

Tumor cell–intrinsic pathways directly blunt T cell activation and recruitment

Oncogenic WNT–β-catenin signaling impairs T cell priming and recruitment

Canonical Wnt–β-catenin signaling, physiologically important for stem cell renewal, has been established as a key driver of cancer progression, and its critical role as a oncogenic driver in many cancers has been well-characterized (Reya and Clevers, 2005; Zhan et al., 2017). An early demonstration that tumor cell–intrinsic aberrant β-catenin signaling could actively modulate the TME to exclude T cells was found in metastatic melanoma. Analysis of metastatic human cutaneous melanoma samples from the Cancer Genome Atlas (TCGA) revealed that patient samples that segregated into the non–T cell–inflamed subset showed enrichment for tumor cell–intrinsic activated β-catenin signaling (Spranger et al., 2015).

A genetically engineered mouse model (GEMM) of melanoma driven by induced expression of active $Braf^{V600E}$ and loss of *Pten* with or without conditional expression of a mutant stable form of β-catenin (BPC and BP mice, respectively) was established to further study the role of β-catenin signaling in T cell exclusion (Spranger et al., 2015). Tumors developed in mice regardless of mutant β-catenin expression, but β-catenin–positive tumors showed a dramatic decrease in T cell infiltration compared with β-catenin–negative tumors. These β-catenin–positive tumors were also resistant to combination anti–CTLA-4 and anti–PD-L1 treatment. Active β-catenin resulted in reduction of chemokine expression, including CC-chemokine ligand (CCL) 4 production, which led to reduced recruitment of a specific subset of DC, the CD103⁺ DC, which are driven by the transcription factor Batf3 (Satpathy et al., 2012) and are able to cross-present (Joffre et al., 2012). These cross-presenting DC are critical in priming the anti-tumor T cell response as they harbor the ability to engulf tumor-derived peptides and via cross-presentation present peptide to naive CD8⁺ T cells (Hildner et al., 2008; Fuertes et al., 2011). In support of the crucial role of cross-presenting DC in initiating the cancer-immunity cycle, intratumoral administration of bone marrow–derived DC in β-catenin–positive tumors led to increased T cell infiltration and greater tumor control and synergized with checkpoint blockade antibody therapy (Spranger et al., 2015). These data illustrate how tumor cell–intrinsic β-catenin signaling can alter the immune composition of the TME to disrupt initiation of the cancer-immunity cycle through impairment of T cell priming.

Subsequent work focused on adoptive T cell transfer of tumor-reactive effector T cells to overcome the lack of T cell activation in β-catenin–positive tumors. However, no tumor control could be observed. Intravital imaging and flow cytometry

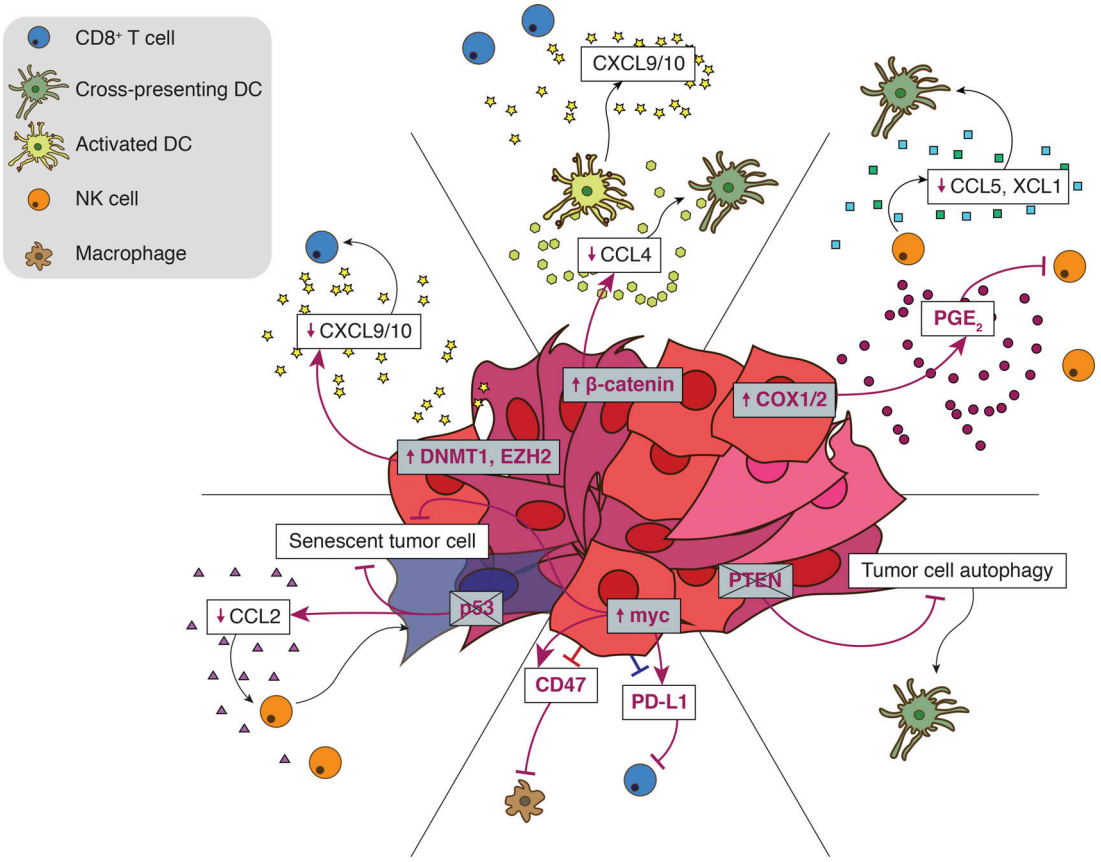

Figure 2. **Tumor cell–intrinsic pathways directly blunting T cell activation and recruitment.** Activated β-catenin signaling leads to a reduction of CCL4 production. CCL4 recruits cross-presenting CD103⁺ DC that are critical for cross-priming CD8⁺ T cells. With the loss of this population from the TME, the cancer-immunity cycle is not initiated. Furthermore, these DC produce the T cell chemoattractant CXCL9 and 10, so there is also loss of T cell recruitment. Elevated COX1/2 activity produces immunosuppressive PGE_2 that can function in a number of different ways. Here PGE_2 is shown to blunt the recruitment and activity of NK cells, leading to a loss of CCL5 and XCL1, chemokines that attract CD103⁺ DC, which lead to loss of T cell priming and recruitment. Loss of p53 results in a reduction of chemokine production by senescent tumor cells, like CCL2, that recruit NK cells to the TME. Moreover, $p53^{-/-}$ tumors also lack production of ligands and cytokines to activate NK cells. The net result is impairment of tumor clearance mediated by NK cells. Loss of PTEN perturbs tumor cell autophagy. Reduction of autophagic activity could abrogate effective T cell priming and is shown to enhance resistance to T cell killing. Oncogenic MYC signaling up-regulates CD47 and PD-L1 on tumor cells. CD47 acts as a "don't eat me" signal, inhibiting phagocytosis by engaging SIRPα on APC, including macrophages, while PD-L1 inhibits T cell function by engaging PD-1 on T cells. Epigenetic remodeling, induced by the activity of methyltransferases DNMT1 and EZH2, suppresses the expression of T cell recruiting chemokines CXCL9 and CXCL10, leading to a reduction in T cell infiltration.

analysis indicated that transferred effector T cells failed to infiltrate BPC tumors compared with BP tumors, demonstrating that in addition to defective priming, oncogenic β-catenin signaling also resulted in impaired T cell recruitment (Spranger et al., 2017). Defective T cell trafficking is largely attributed to loss of T cell–recruiting chemokines CXCL9 and CXCL10 produced by CD103⁺ DC (Spranger et al., 2017). Collectively these studies emphasize the integral role of CD103⁺ DC in initiating and homing the anti-tumor T cell response. These findings in murine melanoma models are supported by studies in human primary melanoma, similarly suggesting that tumor cell–intrinsic activation of the β-catenin pathway is associated with T cell exclusion (Nsengimana et al., 2018).

Further profiling of patient samples available in TCGA revealed that mutations in members of the β-catenin signaling pathway were threefold higher in non–T cell–inflamed tumors than T cell–inflamed samples (Luke et al., 2019). Using different methods to classify β-catenin signaling status, this analysis showed that across 31 solid tumor types, there is a negative correlation between active β-catenin signaling and presence of a T cell–inflamed signature (Luke et al., 2019). This negative correlation of β-catenin expression and CD8⁺ T cell infiltration was also observed in another metastatic melanoma cohort (Massi et al., 2017). These studies highlight a potential role for oncogenic β-catenin signaling to not only promote tumorigenesis but also mediate immune evasion. Further studies are needed to determine whether therapeutic targeting of this tumor cell–intrinsic pathway can indeed induce a T cell–inflamed TME.

Tumor-derived prostaglandin E2 (PGE_2) is an immunosuppressive modulator of cross-presenting DC

Similar to activation of WNT/β-catenin signaling in tumor cells, PGE_2, a pro-inflammatory prostanoid, is suggested to impair anti-tumor immune responses by affecting the CD103⁺–DC–CD8⁺–T cell axis (Wang and Dubois, 2010; Zelenay et al., 2015; Böttcher et al., 2018). While our discussion and the current data suggest an immune

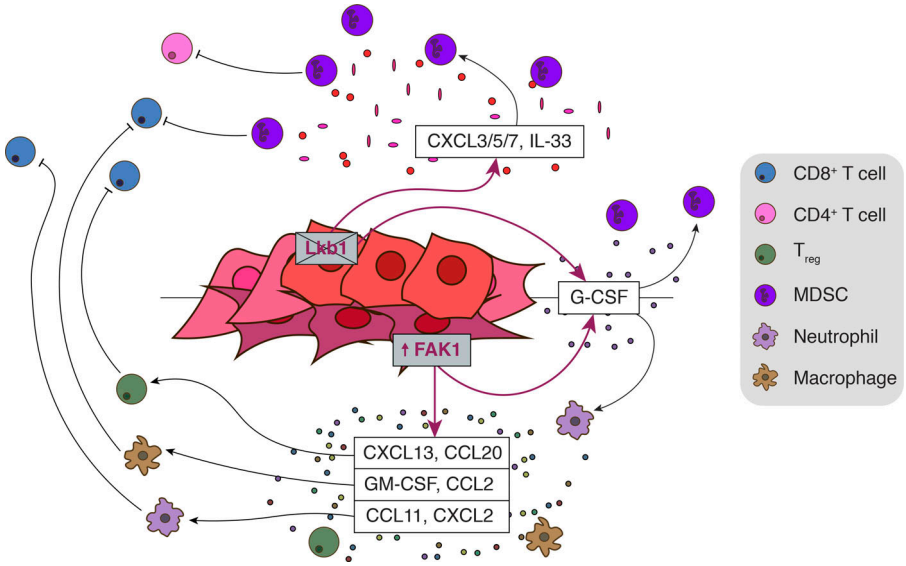

Figure 3. **Recruitment of immunosuppressive populations to the TME.** Loss-of-function LKB1 induces production of cytokines, including CXCL7, IL-33, and G-CSF, leading to neutrophil recruitment. These neutrophils impede T cell trafficking and activity. FAK1 activity promotes transcriptional changes in the tumor cell, inducing gross changes in cytokine production that recruit immunosuppressive populations including macrophages, MDSC, and Treg cells. GM-CSF, granulocyte-macrophage colony-stimulating factor.

suppressive effect of tumor-derived PGE_2 on anti-tumor immunity, it should be noted that stromal cells are also capable of producing this lipid (Kalinski, 2012). Using a transplantable melanoma model with a murine cell line derived from lesions in a GEMM where oncogenesis is driven by expression of active $Braf^{V600E}$ and loss of $p16^{INK4a}$, it was found that PGE_2 produced by the cell line was able to modulate properties of the myeloid cell compartment (Zelenay et al., 2015). Specifically, PGE_2 in conditioned media from the melanoma cell line inhibited lipopolysaccharide-induced production of cytokines like TNFα and interleukin (IL)-12 by myeloid cells. Tumor-derived PGE_2 secretion is dependent on MAPK/ERK signaling (Scherle et al., 1998), and in this study, inhibition of this pathway did result in reduced PGE_2 secretion (Zelenay et al., 2015). It is therefore plausible that Braf and potentially also Kras signaling, two common driver-oncogenes, would result in secretion of PGE_2 (Scherle et al., 1998; Dhillon et al., 2007). This could potentially explain why some melanoma patients being treated with BRAF inhibitors show increased effector immune cell infiltration (Knight et al., 2013).

As PGE_2 production is dependent on cyclooxygenase (COX)–1 and –2 (FitzGerald, 2003), the authors generated a COX-1/COX-2 (*Ptgs1/Ptgs2*) double knock-out (KO) cell line ($Ptgs1/2^{-/-}$ $Braf^{V600E}$) to abolish tumor cell–derived PGE_2 in order to study the effect of tumor cell–intrinsic COX activity (Zelenay et al., 2015). In vitro, the PGE_2-mediated inhibition of cytokine production was largely lost when conditioned media collected from the COX-2–deficient cell line were used to culture myeloid cells (Zelenay et al., 2015). Furthermore, quantitative PCR analysis of an array of immune-related genes of the parental tumor cell line and the COX-deficient derivatives revealed that the COX-deficient lines had drastic changes in global cytokine production, exhibiting a more anti-tumor inflammatory type I immunity gene profile in vivo, including expression of IFN-γ and IFN-stimulated genes, suggesting that PGE_2 suppresses cytotoxic immune responses in the TME (Zelenay et al., 2015).

Examining the immune infiltrates of parental and double KO tumors revealed a striking loss of cross-presenting $CD103^+$ DC in the parental tumors. In the COX-deficient tumors, not only were $CD103^+$ DC present, but a larger fraction of these DC produced IL-12, and all DC subsets displayed higher expression of costimulatory molecules like CD86 and CD40, indicating that these DC were activated (Hubo et al., 2013). These data suggest that the tumor-derived PGE_2 can suppress anti-tumor immunity by suppressing infiltration of $CD103^+$ DC and their activity. This corresponds with previous work, both in vivo with a PGE_2-receptor (EP2) KO mouse model (Yang et al., 2003), and in in vitro studies with bone marrow–derived DC (Sharma et al., 2003) that showed PGE_2 could suppress DC differentiation and function.

Consistent with the findings of increased $CD103^+$ DC infiltration, COX-deficient tumors spontaneously regressed in immunocompetent syngeneic mice in contrast to COX-sufficient control lines, but were able to grow in immunocompromised mice including $Rag1^{-/-}$, which lack B and T cells, $Tap1^{-/-}$ lacking $CD8^+$ T cells, and $Batf3^{-/-}$ lacking $CD103^+$ DC, providing additional evidence that COX activity impairs the $CD103^+$–DC–$CD8^+$–T cell axis (Zelenay et al., 2015). These data were recapitulated with multiple melanoma cell lines, CT26 colorectal, and 4T1 breast cancer cell lines, suggesting that the immunosuppressive activity of PGE_2 may be generalizable across different tumor types (Zelenay et al., 2015).

Other studies have demonstrated additional roles for PGE_2 signaling on immune function, including ovarian cancer studies done with human tissue that showed PGE_2-induced expression of the death receptor Fas ligand (FasL) on endothelial cells could engage Fas on activated $CD8^+$ T cells and specifically mediate effector T cell death (Motz et al., 2014). This phenotype appeared to be coordinated by the coexpression of COX enzymes and VEGF-A by the tumor cells. Treatment of mice bearing ID8-VEGF (Zhang et al., 2002) tumors with blockade of VEGF-A and inhibition of PGE_2 production resulted in a reduction in FasL-positive endothelial cells, an increase in both effector T cell infiltration and the ratio of T effector cells to regulatory T (Treg) cells, and significant tumor control compared with single-agent treatment, suggesting that in this model, both VEGF-A and PGE_2 are important for establishing an immune-suppressive TME

(Motz et al., 2014). In this study, tumor-derived VEGF-A and PGE_2 functioned by paracrine signaling to induce expression of FasL specifically on endothelial cells (Motz et al., 2014), which in part contrasts with the work by Zelenay et al. (2015) that found up-regulation of FasL and Fas in COX-deficient tumors in an analysis performed on bulk tumor tissue. This suggests that the role of PGE_2 in immune evasion is heavily context-dependent and further highlights the complexity of the TME and the challenges in deconvoluting the impact of tumor cell–intrinsic signaling on anti-tumor immune responses.

Administration of acetylsalicylic acid (aspirin) to inhibit COX activity (Vane, 1971; Blobaum and Marnett, 2007) was shown to synergize with anti–PD-1 treatment in mice implanted with $Braf^{V600E}$ melanoma, an effect that was lost in $Rag1^{-/-}$ mice, consistent with the model that COX activity largely suppresses the anti-tumor immune response through adaptive immunity (Zelenay et al., 2015). This study also found that in human melanoma tissue, PTGS2 mRNA expression levels were positively correlated with mRNA expression levels for tumor-promoting inflammatory factors and negatively correlated with CD8A and CD8B mRNA levels, a proxy for presence of a T cell infiltrate. For tumors exhibiting COX activity, inhibition of PGE_2 may be a potentiating factor for patients being treated with checkpoint blockade therapy.

Recent work with this transplantable melanoma model to delineate how PGE_2 could suppress $CD103^+$ DC infiltration showed that natural killer (NK) cells accumulated early on during tumor growth of the COX-deficient $Ptgs1/2^{-/-}$ $Braf^{V600E}$ cell line (Böttcher et al., 2018). Previous studies have shown that PGE_2 can suppress NK cell function (Brunda et al., 1980) by acting on G protein–coupled receptors expressed by NK cells (Holt et al., 2011), suggesting that PGE_2 could limit an immune response by regulating the NK cell compartment. Antibody-mediated depletion of NK cells led to a concurrent reduction of $CD103^+$ DC and $CD8^+$ T cells in the tumors (Böttcher et al., 2018). Additional profiling of the tumors, focused on chemokines expressed by NK cells, revealed that COX-deficient tumors produced higher protein levels of CCL5 and more XCL1 mRNA than control tumors. This difference was also observed in flow cytometry analysis of intratumoral NK cells, indicating they were a source of these chemokines. In vitro–activated splenic NK cells from wild-type mice produced CCL5 and XCL1; however, chemokine production was reduced in a dose-dependent manner upon culture with PGE_2. Interestingly, PGE_2 also reduced NK cell survival. NK cells derived from COX-deficient tumors maintained this susceptibility to PGE_2 treatment. Antibody-mediated blocking of CCL5 and XCL1 in wild-type mice before implantation of the COX-deficient lines led to a significant reduction of $CD103^+$ DC while overexpression of either cytokines resulted in an increase of these cross-presenting DC and accelerated rejection compared with control lines. These data provide evidence that tumor-derived PGE_2 can directly modulate NK cell activity and thereby affect other immune cell types such as DC and T cells (Harizi, 2013; Crouse et al., 2015). Recent work focusing on human malignant melanoma has highlighted that, in fact, properly activated NK cells produce FMS-like tyrosine kinase 3 ligand in situ and thereby enhance the presence of $CD103^+$ DC within the TME (Barry et al., 2018).

In sum, these data suggest that PGE_2, often induced via COX signaling activation, blunts the cancer-immunity cycle at the state of sensing and homing by excluding innate immune cell types including NK cells and DC. However, it still needs to be determined if NK cells are critical for this mechanism across different tumor types.

Loss of p53 activity reduces NK cell recruitment and activation

The tumor suppressor p53 is a key regulator of cell-cycle progression. p53 acts as a safeguard against cell division in the presence of a multitude of different stressors, sometimes inducing cell death or senescence, thus preventing an accumulation of cells that could become cancer. Astoundingly, p53 is mutated in ∼50% of all cancers, emphasizing its anti-cancer function (Soussi and Wiman, 2007). In an early study, the role of p53 after late-stage tumor establishment was investigated in a mouse liver cancer model with inducible restoration of p53 expression (Xue et al., 2007). Even transient induction (4 d) of p53 expression was sufficient to drive complete tumor regression in orthotopic and subcutaneous implantations. This pulse of p53 activity resulted in senescence of the tumor cells rather than apoptosis. These tumors were found to be infiltrated by neutrophils, macrophages, and NK cells. Depletion of any of these populations resulted in delayed tumor regression, suggesting some coordination of all three to more effectively drive tumor control.

Using the same model, another group discovered that p53 restoration resulted in up-regulation of chemokines and cytokines including CCL2, CCL3, CCL4, CCL5, CXCL1, and CXCL2 among many others (Iannello et al., 2013). CCL2 appeared to be the dominant signaling molecule with the most dramatic effect on NK recruitment. These data illustrate how restoration of a key tumor suppressor can remodel the TME to establish a productive immune response, this time acting on NK cells. A recent study showed that in mouse transplant models of lymphoma and melanoma, activation of p53 with pharmacological treatment could induce systemic anti-tumor immunity, leading to regression of immunogenic tumors (Guo et al., 2017). Efficacy of treatment was contingent upon induction of an immune-stimulatory TME and immunogenic cell death (Kroemer et al., 2013; Kepp et al., 2014; Galluzzi et al., 2017) mediated by activated p53 (Guo et al., 2017). These studies also suggest that immune evasion can be established early during tumorigenesis as p53 is often lost early on as a driver for cancer development. Additional studies will be needed to further elucidate the immunological mechanisms of this model, especially given the recent evidence that NK cells can affect recruitment of DC into the TME with significant effects on T cell activation and recruitment.

Loss of phosphatase and tensin homologue (PTEN) suppresses T cell mediated killing and T cell infiltration

Alterations in the phosphatidylinositol 3-kinase (PI3K)/PTEN pathway are frequent pro-oncogenic events contributing to cancer progression and proliferation (Chalhoub and Baker, 2009). PI3K activation upon response to growth factors leads to activation of protein kinase B (Akt), which induces a pro-survival and proliferative cellular program, while the tumor

suppressor PTEN reduces activation of Akt by antagonizing PI3K activity (Chalhoub and Baker, 2009). Loss of PTEN found with an activating BRAF mutation is a frequent occurrence in melanoma patients (Akbani et al., 2015). Analyses of cancer cohorts have shown that PTEN loss and oncogenic BRAF signaling are associated with poorer prognosis (Peng et al., 2016). Recent insights gained from a study of 135 patients with resected melanoma regional metastases (Bucheit et al., 2014) suggested that PTEN loss was correlated with a significant reduction of $CD8^+$ T cell infiltration when compared with PTEN sufficient tumors (Peng et al., 2016).

Additional analyses of TCGA datasets revealed that tumors with low PTEN copy numbers also had lower expression of LCK, a protein largely expressed by T cells, and lower transcripts of the T cell–effector molecules IFN-γ and granzyme B (Peng et al., 2016). These observations possibly reflect a lack of T cell infiltration in these patient samples. Accordingly, segregating TCGA samples into T cell–inflamed and non–T cell–inflamed groups revealed a higher frequency of loss of function PTEN mutations and gene deletions in the non–T cell–inflamed group than the T cell–inflamed group (Peng et al., 2016). Of note, it did not appear that there is a significant overlap of activating β-catenin mutations in this group, suggesting that loss of PTEN may be an independent tumor cell–intrinsic event that excludes T cells from the TME.

To obtain mechanistic insight, a transplant model was developed using the human melanoma line A375 to follow up on the clinical findings. The human melanoma cell line was transduced to stably express the mouse major histocompatibility complex I (MHCI) molecule $H\text{-}2D^b$ and the melanoma tumor antigen gp100, yielding a line that can be recognized by antigen-specific T cells from T cell receptor (TCR) transgenic PMEL mice (Abad et al., 2008). In vitro co-culture assays of the tumor cells and PMEL T cells resulted in a reduction in killing of tumor cells when PTEN was knocked down, indicating that PTEN-deficient tumor cells are more resistant to T cell–mediated lysis (Peng et al., 2016). In vivo, the PTEN-deficient tumors also showed a reduction in accumulation of transferred PMEL T cells compared with the control tumors. Accordingly, the therapeutic effect of this therapy was reduced in mice bearing PTEN-deficient tumors, resulting in reduced tumor control and a reduction in percent survival compared with mice with control tumors. Knockdown of PTEN was not associated with changes in PD-L1 or MHCI, suggesting a different mechanism for immune evasion.

Differential gene analysis performed on PTEN-deficient and control tumors showed that CCL2 and VEGF were significantly up-regulated in PTEN-deficient tumors (Peng et al., 2016). CCL2 and VEGF have been ascribed multiple functions including recruitment of immunosuppressive cell populations like macrophages, MDSC, and Treg cells (Nagarsheth et al., 2017; Yang et al., 2018). More extensive phenotyping of this model paired with modulation of these signals would determine if these signals contribute to a shift in the immune populations that would promote tumorigenesis.

ATG16L, a component of a ubiquitin-like protein conjugation system critical for elongation of the preautophagosomal membrane (Kuang et al., 2013; Xiong et al., 2018), was identified when analyses were performed to identify tumor-specific gene expression changes in human melanoma cell lines with or without silenced PTEN (Peng et al., 2016). ATG16L was up-regulated in tumors with PTEN. Perturbing autophagy genes in co-culture assays revealed that overexpression of autophagy genes in tumor cells enhanced T cell–induced apoptosis while knockdown resulted in resistance to T cell killing. A previous study showed that in vivo autophagy, induced by chemotherapy, was required for tumor immunogenicity (Michaud et al., 2011) during immunogenic cell death (Kepp et al., 2014; Galluzzi et al., 2017). This was an effect mediated by immunogenic ATP release from dying cells and required DC and T cells (Michaud et al., 2011). Consistently, other groups have found that dying cells exhibiting enhanced autophagy allow more robust priming of $CD8^+$ T cells (Uhl et al., 2009), while work using different models identified that inhibition of the early stages of autophagy results in a significant reduction of cross-presentation by DC and thereby reduced T cell activation (Li et al., 2008). More work will be needed to fully elucidate the effects of increased autophagy on T cell activation and DC-mediated T cell priming.

Since loss of PTEN resulted in elevated levels of phosphorylated-AKT in this melanoma xenograft model, PI3K was targeted to regulate phosphorylated-AKT in tumor cells. Inhibition of the PI3Kβ isoform, which is dispensable in TCR signaling, synergized with anti–PD-1 checkpoint blockade therapy to treat mice with sizeable PTEN-deficient autochthonous melanoma (Peng et al., 2016). The data suggest that targeting the PI3K-Akt pathway may be beneficial for melanoma patients with PTEN loss. Recent preclinical models of melanoma exhibiting PTEN loss showed that a combination treatment of an agonist targeting a T cell costimulatory molecule (OX40; Sugamura et al., 2004; Croft et al., 2009), with GSK2636771 (Mateo et al., 2017), a PI3Kβ inhibitor, could control tumor growth and extend survival in mice by enhancing the anti-tumor immune response through expansion of tumor-infiltrating $CD8^+$ T cells (Peng et al., 2019). The current clinical trial NCT03131908 (https://clinicaltrials.gov/ct2/show/NCT03131908) is recruiting patients with refractory metastatic melanoma to administer a combination therapy of GSK2636771 with pembrolizumab, an anti–PD-1 checkpoint blockade antibody, to determine the efficacy of this treatment.

Oncogenic MYC up-regulates PD-L1 and CD47 on tumor cells to evade T cells

The transcription factor MYC is a master regulator of cellular proliferation and differentiation, and its activation by overexpression is a common occurrence in many cancers (Dang, 2012). A recent analysis of TCGA revealed that gene amplification of *Myc* paralogs occurs in 28% of samples analyzed, spanning the 33 solid cancer types available in TCGA, emphasizing the importance of this genetic alteration in tumorigenesis (Schaub et al., 2018).

Previously, it was believed that the phenomenon of oncogene addiction, wherein tumor cells undergo regression through growth arrest, apoptosis, and senescence, was a tumor cell–autonomous response to oncogene inactivation (Weinstein and

Joe, 2008). A major finding using transplants derived from a GEMM of MYC-induced T cell acute lymphoblastic lymphoma (MYC T-ALL) revealed that CD4+ T cells were necessary for complete and durable tumor regression (Rakhra et al., 2010). More specifically, CD4+ T cells were needed for the induction of cellular senescence and angiogenesis inhibition upon *Myc* inactivation. This work provided evidence that the immune system is a key extrinsic factor of oncogene addiction.

Myc inactivation led to an increase in Thrombospondin-1 (TSP-1) expression in the TME (Rakhra et al., 2010). TSP-1 is an anti-angiogenic cytokine (Lawler and Lawler, 2012). Interestingly, tumors transplanted in RAG2$^{-/-}$ and CD4$^{-/-}$ host mice showed no up-regulation of TSP-1, suggesting an indirect effect of T cells (Rakhra et al., 2010). CD4+ T cells also express TSP-1, and reconstitution experiments revealed that TSP-1 expression in immune cells was needed for tumor control following *Myc* inactivation. TSP-1 overexpression directly in the tumor cells could overcome the requirement for T cells for the inhibition of angiogenesis.

A subsequent study using the MYC T-ALL GEMM provided additional evidence of the immune suppressive impact of tumor cell–intrinsic MYC signaling (Casey et al., 2016). MYC expression was found to induce expression of PD-L1 and CD47, which was reduced following therapeutic MYC inhibition. PD-L1 engagement with PD-1 on T cells results in attenuation of TCR signaling while CD47 binds to SIRPα, which is found on phagocytic cells like macrophages, inhibiting phagocytosis (Jaiswal et al., 2009; Majeti et al., 2009). When MYC was targeted with shRNA or the bromodomain-extraterminal inhibitor JQ1 in human T-ALL cell lines with myc amplification, there was a decrease in CD47 and PD-L1 expression (Casey et al., 2016). Chromatin-immunoprecipitation sequencing experiments identified high MYC binding to the promoter regions of CD47 and PD-L1, suggesting that MYC is a transcriptional regulator of these genes (Casey et al., 2016).

Another transplant model used the MYC T-ALL 4188 cell line, which recruits immune cells when *Myc* is inactivated, to show that forced overexpression of either PD-L1 or CD47 was sufficient to suppress the recruitment of immune effectors that included T cells and F4/80+ macrophages (Casey et al., 2016). The 4188 line overexpressing either molecule also showed tumor progression in vivo compared with the parental line when *Myc* was inactivated. The dramatic loss of effector T cells, specifically the CD4+ T cells that were the source of anti-angiogenic TSP-1, following overexpression of either checkpoint molecule could partly explain how stabilization of angiogenesis and loss of cellular senescence are achieved. It needs to be pointed out that the mechanism of how PD-L1 and CD47 achieve these effects is still unknown as neither molecule has been shown to have a direct impact on immune cell recruitment.

Epigenetic marks mediate down-regulation of immune-stimulatory cytokines

Changes in the epigenomic landscape have a major impact on tumorigenesis (Subramaniam et al., 2014; Flavahan et al., 2017; Jones et al., 2018), but only recently has evidence for the influence of epigenetic marks on anti-tumor immune responses been provided. In ovarian cancer, the treatment of tumors with methyltransferase inhibitors targeting EZH2 (a histone methyltransferase) and DNMT1 (a DNA methyltransferase) led to tumor reduction (Peng et al., 2015). Tumor control was associated with enhanced CXCL9 and CXCL10 expression by tumor cells, which in turn mediated an increase in recruitment of CD8+ effector T cells. Tumor control following methyltransferase inhibition was lost in immune-deficient NSG mice, providing evidence that the adaptive immune response, presumably T cells, played an essential role in controlling tumor progression following epigenetic reprogramming. The study performed extensive epigenetic profiling of human primary and established ovarian cancer cell lines. In one experiment, chromatin-immunoprecipitation sequencing following EZH2 inhibition showed loss of repressive H3K27me3 marks at the promoters of CXCL9 and 10, and accordingly there was higher expression of IFNγ-induced CXCL9 and 10 in treated cells. Inhibition of EZH2 did not affect DNMT1 expression, and likewise inhibition of DNMT1 did not affect H3K27me3 marks. Moreover, inhibition of one methyltransferase in a cell line where the other enzyme has been knocked down enhanced CXCL10 expression, revealing a certain degree of independence between EZH2 and DMNT1 function at regulating the particular loci of interest, which could explain why single-agent treatment was not efficacious. The immune-stimulatory changes induced in the TME dual-inhibitor treatment were highly synergistic with immunotherapeutic treatments (Peng et al., 2015). Another group has provided evidence from murine melanoma models that the resulting immune response after immunotherapy-induced up-regulation of EZH2, resulting in gene expression changes that promoted immune evasion (Zingg et al., 2017). In these models, EZH2 inhibition synergized with immunotherapy and reversed the adaptive resistance (Zingg et al., 2017). It is an intriguing concept that some patients experiencing progressive disease following an initial response to immunotherapy could benefit from targeted modulation of epigenetic regulators.

Analyses of ovarian cancer patient samples found that overall survival along with disease-free interval were shorter in patients with either high expression of EZH2 or DNMT1; these prognostic values were further augmented when looking at patients with high expression of both proteins versus looking at each independently (Peng et al., 2015). CD8+ T cell infiltration positively correlated with survival and negatively correlated with expression of EZH2 and DNMT1 (Peng et al., 2016). The data offer evidence that tumors can take advantage of epigenomic alterations, a hallmark of cancer, to evade the immune system. Using a GEMM to induce autochthonous tumors would allow for a more progressive look at how the epigenome evolves over time and the parallel changes in the TME. More specifically, it would provide a more detailed look at when these particular marks are made, offering insight into when immune evasion takes place. Comparing tumors derived from different time points might also reveal the mechanisms driving the placement of these marks. Further studies are needed to integrate chemokine production by the tumor with production by immune cells residing within the TME and to elucidate the functional consequences of the specific source of chemokines for downstream effects. These insights would be valuable for designing rational treatment strategies.

Recruitment of immunosuppressive populations

Loss-of-function LKB1 results in recruitment of immunosuppressive neutrophils

The tumor suppressor LKB1 is a serine/threonine kinase that phosphorylates AMPK, a kinase that regulates metabolism (Shackelford and Shaw, 2009). This pathway is activated when nutrient levels are low to dampen cellular growth and proliferation. Loss-of-function mutations in LKB1 are found in approximately one third of Kras-driven lung adenocarcinoma and have been linked with a more aggressive tumor progression phenotype (Calles et al., 2015), though it was mostly unclear whether LKB1 could directly impact anti-tumor immunity.

Using a GEMM of non-small cell lung cancer, driven by Cre-inducible expression of mutant $Kras^{G12D}$ in combination with conditional LKB1 loss, it was possible to determine that LKB1 loss was associated with infiltration of $CD11b^+$ $Ly\text{-}6G^+$ neutrophils (tumor-associated neutrophils [TAN]) into the tumor (Koyama et al., 2016). This infiltration was not observed in tumors with wild-type LKB1 and was correlated with increased neutrophil counts in peripheral blood and spleen, indicating greater global expansion of neutrophil populations in mice bearing LKB1-deficient tumors.

Analysis of the transcriptome of isolated tumor cells revealed that LKB1-deficient tumors express higher levels of CXCL3, CXCL5, and CXCL7—all chemokines that recruit CXCR2-expressing neutrophils (Griffith et al., 2014), along with granulocyte colony-stimulating factor (G-CSF), IL-33, and IL-1α. Additionally, detailed analysis of TAN sorted from the LKB1-deficient and proficient tumors provided evidence that TAN isolated from LKB1-deficient tumors expressed higher levels of proinflammatory IL-6, a cytokine associated with wound repair responses (Gallucci et al., 2000; Lin et al., 2003). These tumors also demonstrate a lower degree of both $CD4^+$ and $CD8^+$ T cell infiltration. T cells found in these tumors express higher levels of checkpoint molecules like PD-1 and CTLA-4 compared with the T cells found in control tumors, suggesting that the T cells found in the LKB1-deficient tumors are indeed tumor-reactive. At the same time, the T cells from these tumors also expressed lower levels of Ki-67 and IFN-γ (Koyama et al., 2016), indicating a decrease in proliferation and effector function, respectively, suggesting an exhausted or dysfunctional T cell phenotype (Horton et al., 2018).

To further probe the role of the TAN in tumorigenesis, an anti–Ly-6G antibody was used to deplete TAN in mice with established Lkb1-deficient tumors (Koyama et al., 2016). After treatment, there was a reduction of TAN, IL-6, and G-CSF expression and an increase in total $CD8^+$ T cell numbers as well as an increase in Ki-67 and IFN-γ staining of these T cells. Depletion of TAN resulted in a reversal of the phenotype associated with LKB1 loss-of-function, providing evidence that the TAN accumulation mediated by loss of LKB1 directly blunts anti-tumor T cell responses. Similar to TAN depletion, treatment with a neutralizing IL-6 antibody mediated tumor control in a T cell–dependent manner and specifically enhanced anti-tumor immunity by restoring T cell proliferation and function. Anti–PD-1 checkpoint blockade therapy in this model was not efficacious, and no synergistic effect with anti–IL-6 blocking antibody was observed.

Knock-down of LKB1 in human non-small cell lung cancer cell lines confirmed that IL-6, G-CSF, and CXCL7 production were indeed enhanced following LKB1 loss (Koyama et al., 2016). Additionally, loss of LKB1 was associated with a reduction of PD-L1 expression, which might explain why anti–PD-1 checkpoint blockade is ineffective in this model. This highlights the translational relevance of those findings but also the complex and multifold impact of LKB1 loss on tumor cells and immune responses. Further, these experiments provide additional evidence that this modulation of PD-L1 expression is cell intrinsic and does not completely depend on extrinsic factors, like IFN-γ production by T cells (Dong et al., 2018). The inverse correlation between PD-L1 and loss of LKB1 was corroborated in analyses of lung cancer cell lines from TCGA and in the MD Anderson Cancer Center PROSPECT cohort (Koyama et al., 2016). Further, immunohistochemistry (IHC) staining revealed that wild-type LKB1-proficient tumors had significantly higher numbers of $CD8^+$ T cells compared with LKB1-deficient specimens. These analyses support the notion that LKB1 loss may play an important role in immune evasion and escape in lung cancers. More work will be necessary to fully elucidate how TAN influences T cell trafficking and activity. The data suggest that modulation of neutrophils directly would increase anti-tumor T cell responses; however, it needs to be further understood why this increase does not synergize with checkpoint blockade immunotherapy.

Focal adhesion kinase activity drives induction of an immunosuppressive TME

Analyses of patient pancreatic tumor tissue have shown that T cells can infiltrate pancreatic lesions and furthermore, in line with observations made in other cancer types, T cell infiltration also correlates with response to checkpoint blockade therapy in pancreatic ductal adenocarcinoma (PDAC; Torphy et al., 2018). Previous studies using GEMMs to model PDAC have characterized the resulting tumors as containing densely fibrotic stroma and immunosuppressive immune cell populations, namely, tumor-associated macrophages (TAM), MDSC, and Treg cells (Clark et al., 2007; Feig et al., 2012; Jiang et al., 2016).

FAK have been implicated in a number of different diseases where fibrosis is a hallmark of disease (Lagares and Kapoor, 2013). FAK are pleiotropic nonreceptor tyrosine kinases that regulate many different cell processes including adhesion, migration, and proliferation and been found to be up-regulated in a number of different cancers, including pancreatic cancer (Parsons et al., 2008). A study found that ∼80% of patient samples analyzed exhibited higher expression of FAK1 and higher levels of activated phosphorylated FAK1 (p-FAK1) compared with normal pancreatic tissue (Jiang et al., 2016). Further IHC analysis revealed a negative correlation between tumor p-FAK1 levels and $CD8^+$ T cell infiltration; using these two markers to look at overall survival, the study found that patients with high p-FAK1 and low $CD8^+$ T cell levels showed markedly poor outcome compared with all other patients (Jiang et al., 2016).

A GEMM modeling PDAC in this study driven by pancreatic-specific expression of mutant Kras and loss of p53 revealed low expression of p-FAK1 levels in normal and early neoplastic

lesions, which were markedly increased in late neoplastic lesions and PDAC tissues. Additionally, the TME of PDAC lesions with high p-FAK1 levels exhibited heavy infiltration of granulocytes and TAM, suggesting FAK1 has a function in establishing an immunosuppressive TME (Jiang et al., 2016).

A clinical-grade FAK inhibitor that targets both FAK1 and FAK2 was used to treat the GEMM during early and late stages of tumor burden, and interestingly, in both cases, inhibition of FAK was able to significantly prolong survival to a similar degree, suggesting that FAK also has a critical role in maintaining the immunosuppressive TME (Jiang et al., 2016). Mice treated with the inhibitor showed a marked decrease in collagen deposition, corresponding with decreased Ki67$^+$ staining in the stroma, indicating that FAK inhibition could deplete fibrotic stroma. In contrast to other findings where disease progression accelerated upon stromal depletion (Rhim et al., 2014; Özdemir et al., 2014), this study found a decrease in numbers of invasive cells, tumor-initiating cells, and liver metastasis along with the decrease in fibrotic stroma in mice treated with the FAK inhibitor (Jiang et al., 2016).

IHC analyses of the PDAC tissue showed that the treated mice had fewer infiltrating TAM and granulocytes including MDSC (Jiang et al., 2016). Similarly, reduction of tumor-infiltration Treg cells was also observed. The evidence strongly supports the notion that FAK signaling could facilitate the induction of an immune-suppressive TME.

To dissect whether p-FAK1 expressed in stroma or tumor cells mediated the immune suppression, orthotopic tumor models with genetic FAK depletion were employed (Jiang et al., 2016). Tumor cell ablation of FAK1 was found to be sufficient to reduce collagen deposition and suppressive myeloid cell populations while increasing CD8$^+$ T cell infiltration. FAK inhibition was associated with decreased production of pro-fibrotic and pro-inflammatory cytokines. Cancer-associated fibroblasts (CAFs; Kalluri, 2016; Yamauchi et al., 2018; Stromnes et al., 2014) are a major component of the tumor stroma in PDAC that drive the remodeling of the fibrotic ECM by excessive production of ECM components such as collagens (von Ahrens et al., 2017; Qu et al., 2018; Stromnes et al., 2014). Since tumor cell–specific FAK1 depletion resulted in reduced collagen deposition, the authors assessed the ability of PDAC cells to promote CAF proliferation and identified that FAK1-deficient tumor cells induced less CAF proliferation compared with FAK1-proficient tumor cells (Jiang et al., 2016). Neutralization of CXCL12 (Strieter et al., 2007) in FAK1-proficient tumor conditions resulted in reduction of CAF proliferation rates to similar rates observed in FAK1-deficient tumor, suggesting that FAK1 activity can drive production of pro-fibrotic factors such as CXCL12 that induce stromal expansion (Jiang et al., 2016). Intriguingly, the authors identified that increased collagen density could induce FAK activation, indicating a positive feedback loop between PDAC cells and CAFs.

FAK inhibitor treatment also resulted in decreased production of pro-inflammatory cytokines involved in recruiting macrophages, granulocytes, and Treg cells. A previous study of FAK activity in squamous cell carcinoma found that nuclear FAK could regulate transcription of chemokines and cytokines implicated in recruitment and expansion of Treg cells, suggesting a related mechanism of action for FAK-induced transcriptional changes in the PDAC model (Serrels et al., 2015).

It was also observed that PDAC tumor cells in mice treated with a FAK inhibitor showed lower levels of phosphorylated-STAT3 (Jiang et al., 2016). In an inducible PyVmT mouse mammary tumor model, deletion of Stat3 resulted in a delay in tumor onset and establishment of tumors that rapidly regressed (Jones et al., 2016). Stat-deficient tumors exhibited an increase in CD103$^+$ DC along with CD8$^+$ T cells at early time points, suggesting that Stat3 plays an important role in establishing a suppressive TME during early tumorigenesis (Jones et al., 2016). Another study using a mammary tumor model directly showed that FAK disruption impaired tumorigenesis (Lahlou et al., 2007), providing a potential model that explains how FAK activity mediates suppression of anti-tumor immunity beyond PDAC. Consistently, activated STAT3 signaling in PDAC epithelium has also been shown to promote tumor progression by increasing fibrosis and stromal stiffening (Laklai et al., 2016). Increased tension between tumor cells resulted in elevated FAK1 signaling, increased phosphorylated-STAT3 and increased fibrosis, which were again reduced following FAK1 inhibition (Laklai et al., 2016). Further evidence reinforces the relationship between CAF, STAT3 signaling, and PDAC progression as a recent study elucidated that CAFs induce PDAC tumor cell–intrinsic STAT3 signaling, resulting in a more invasive and proliferative phenotype (Ligorio et al., 2019). These studies reveal the tight association between stroma and tumor in promoting tumor progression and support the notion that a fibrotic stroma can contribute to exclusion of cytotoxic T cells.

Because inhibition of FAK activity depleted the stroma and tipped the TME toward a more productive anti-tumor response, combination therapies of FAK inhibition with chemotherapy as well as immunotherapy were investigated. In both orthotopic and autochthonous tumors, gemcitabine and checkpoint blockade therapy synergized with FAK inhibition to reduce tumor burden and prolong survival (Jiang et al., 2016). These findings provide a strong rationale for use of FAK inhibitors in conjunction with standard chemotherapeutics and immunotherapies. However, further studies are needed to fully elucidate which therapeutic combination synergizes under which conditions.

Discussion

The studies selected for discussion in this review represent a small sampling of the ongoing work to understand the impact of tumor cell–intrinsic alterations on anti-tumor immunity. The works selected illustrate the previously unrecognized immune modulatory role of pathways known to drive cancer progression. Additional studies continue to reveal other tumor cell–intrinsic signaling pathways influencing anti-tumor immune responses. For instance, the Hippo pathway, well-characterized as the regulator of organ size and a known contributor to cancer progression (Pan, 2010), has recently been shown to mediate immune suppression by expression of PD-L1 in human breast cancer cell lines (Janse van Rensburg et al., 2018) and BRAF inhibitor–resistant human melanoma lines (Kim et al., 2018). Evidence from a preclinical prostate adenocarcinoma model

suggests that activation of the Hippo pathway and its downstream effector YAP increases MDSC populations (Wang et al., 2016). This along with other examples emphasizes the necessity of reexamining the immune component in cancer models, especially those treated with therapies that are not immediately or previously considered immunomodulatory. An example of unexpected immunomodulation was observed in treatment of a mouse model of breast carcinoma with CDK4/6 inhibitors (Goel et al., 2017). Treatment elicited an anti-tumor immune response that was attributed to enhanced tumor antigen presentation and selective suppression of Treg cell proliferation that increased the $CD8^+$ ratio of T effector cells to Treg cells. Efficacy of treatment was further enhanced with checkpoint blockade therapy.

The complexity of these altered pathways and their potential to alter the immune response in a multitude of ways is exemplified by oncogenic MYC signaling. In the study by Casey et al. (2016), MYC amplification was found to induce expression of PD-L1 and CD47, probably by MYC acting as a transcription factor of these genes, which led to a decrease in recruitment of effector T cells. A previous study using a GEMM of pancreatic β-cell carcinoma revealed rapid induction of cytokines including CCL2, CXCL2, CCL7, and CCL5 that attract immunosuppressive populations including macrophages, neutrophils, and mast cells following *Myc* activation (Soucek et al., 2007). The mast cells were critical for tumor development as abrogating mast cell function prevented expansion of cancerous lesions, providing early evidence of immune function impacting tumorigenesis. More recently, MYC has been shown to accelerate the invasive phenotype of lung adenocarcinoma in a GEMM driven by mutant $Kras^{G12D}$ (Kortlever et al., 2017). It was shown that MYC upregulated CCL9 and IL-23, which led to drastic remodeling of the TME with CCL9 shown to recruit macrophages that promote angiogenesis and contribute to a reduction of T cells while IL-23 mediated exclusion of B, T, and NK cells (Kortlever et al., 2017). Additional studies of murine lung cancer models have also shown that epigenetic changes can influence MYC function. Combination epigenetic treatment, targeting DNA methyltransferase and histone deacetylase in one model, led to depletion of MYC, resulting in release of repression of CCL5 expression, a change that led to increased trafficking of $CD8^+$ T cells into tumors (Topper et al., 2017). This examination of MYC activity in different tumor types characterizes the extraordinary task of parsing the contribution of often pleiotropic gene alterations in tumor–host interactions. In this example, not only does MYC have different effects, shared changes in different models result in different phenotypes. For example, in the murine pancreatic β-cell carcinoma model, CCL5 induced by MYC activation was shown to recruit the pro-tumor mast cells, whereas in epigenetic treatment of murine lung cancer, CCL5 expression was shown to be repressed by MYC and functioned to attract anti-tumor effector T cells. Context is paramount.

The intricate nature of tumor cell–intrinsic signaling has major implications for treatment. A broader understanding of a patient's disease genetically and phenotypically is critical to developing rational combination therapies. Furthermore, mechanistic insight into the tumor-immune dynamics could reveal novel targets to shift or establish a TME that elicits a productive anti-tumor response. The relative ease of developing GEMM that better model patient disease paired with advancing technology, including single-cell sequencing, that allows us greater resolution of the tumor milieu endow us with increasing capacity to delineate how tumor cell–intrinsic genomic alterations can impact the immune system over space and time. A robust approach to these studies holds the promise of bridging the gap between responders and nonresponders of current immunotherapies.

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