B Cells and Melanoma Pathogenesis

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Melanoma is one of the deadliest cancers. In this issue, Kobayashi et al. (2019) demonstrate a novel role for tumor-infiltrating innate-like B1a B cells in promoting melanoma growth. IL-10− (B1a) regulatory B cells accumulate selectively in melanomas, and enhance tumor growth through suppression of cytokine production by tumor-infiltrating CD8+ T cells and potentially additional IL-10—dependent mechanisms.


INTRODUCTION

Melanoma represents only 1% of all skin cancers, but it is one of the deadliest cancers worldwide and is responsible for the majority of skin cancer deaths (Schadendorf et al., 2015). In recent years, great progress has been made in understanding the regulation of melanoma tumor immunity and pathogenesis, leading to the development and approval of new therapies. Recently approved treatments target mutations in different signaling pathways of melanoma cells and inhibit crucial immune checkpoints that harness T cell responses, such as CTLA-4 and PD1, resulting in improved overall survival of melanoma patients (Pelser and Amaria, 2019). However, these treatments are not uniformly effective and novel immunotherapeutic approaches are needed. Understanding the mechanisms of immune evasion in melanoma is paramount and may open new avenues for future approaches to treat this disease.

Regulatory B cells attenuate immunity to melanoma

The involvement of B cells in cutaneous immune responses and cancers is an emerging field (Debes and McGettigan, 2019; Egbuniwe et al., 2015). Kobayashi et al. (2019) investigate the contribution of regulatory B cells to the tumor microenvironment and elucidate their role in modifying melanoma immunity and tumor growth using the B16F10 melanoma mouse model and mice with a B cell–specific PTEN deficiency (Cd19CrePTEnloxp/loxp mice). These mice have greatly expanded IL-10+ B1a B cells. B1a B cells are innate-like B cells that localize to body cavities, skin, and mucosal barriers and show enhanced T cell—dependent antibody responses (Baumgarth, 2011; Debes and McGettigan, 2019).

This exciting study showed that in melanomas of both control and B cell PTEN-deficient mice, the majority of tumor-infiltrating B cells had an innate-like B1a phenotype and secreted IL-10 upon stimulation. Consistent with expansion of B1a B cells in the periphery, melanomas in B cell PTEN-deficient mice contained greater numbers of IL-10+ B1a regulatory B cells, a finding that was associated with accelerated tumor growth. Mechanistically, the authors identified reduced intratumoral CD8+ T cell responses because decreased percentages of these cells expressed IFN-γ and TNF-α in tumors of B cell PTEN–deficient mice. The authors went on to show that the melanoma growth-promoting role for B1a cells in the tumor microenvironment was IL-10—dependent (summarized in Figure 1) by demonstrating that B1a cells from B cell PTEN-deficient or wild-type mice, but not non-B1a B cells or B1a B cells from Il10−/− mice accelerated tumor growth when combined and co-transferred with tumor cells.

In addition to effects on cytokine expression by CD8+ T cells, IL-10 has immunosuppressive effects on many immune cells, ranging from promotion of myeloid-derived suppressor cells to downregulation of antigen-presentation (Figure 1). While other studies have demonstrated a tumorigenic role for IL-10 regulatory B cells in various tumors, such as breast cancer (Chiaruttini et al., 2017), the current study describes regulatory B cell—mediated immunosuppression within the tumor microenvironment itself.

A dual role for B cells in melanoma and other skin cancers

Whereas the study by Kobayashi et al. (2019) suggests an important role for one subset of B regulatory cells in weakening tumor immunity to melanoma, other studies in humans and mouse models of cutaneous cancers have identified both pro- and anti-tumorigenic roles for B cells (reviewed in Chiaruttini et al., 2017; Egbuniwe et al., 2015). B cell infiltration in primary melanomas has even been correlated with a better prognosis for patients characterized by reduced tumor recurrence and visceral metastasis (Chiaruttini et al., 2017). How can B cells promote anti-melanoma responses in some cases, but inhibit tumor immunity and accelerate tumor growth in others? Part of the answer may lie in the existence of different B cell subsets that are typified by different and sometimes opposing functions. Consistent with this, Kobayashi et al. (2019) found both B1a and non-B1a B cells within the tumors, but only B1a B cells could produce IL-10 (Kobayashi et al., 2019).

One mechanism by which B cells promote tumor immunity involves production of tumor-specific IgG (Chiaruttini et al., 2017). However, there are likely to be several mechanisms by which B cells promote tumor immunity in melanoma and other skin cancers. Lessons learned in inflammatory skin diseases about the...
mechanisms by which B cells engage in skin-localized responses (reviewed in Debes and McGettigan, 2019) can likely be applied to cutaneous tumors. Effector functions of cutaneous B cells include secretion of immunostimulatory cytokines, such as GM-CSF and IL-6, antigen-presentation, and activation of skin-resident effector T cells, and establishment of tertiary lymphoid organ structures that further enhance antibody and T cell responses (Debes and McGettigan, 2019). There is indeed some evidence for tertiary lymphoid organ structures in melanoma (Chiaruttini et al., 2017).

Open questions and future directions
Future studies will be important to delineate B cell subsets infiltrating human tumors and to identify strategies that selectively target specific B cell subsets or functions rather than B cells overall. As an example, for such B cell subset—specific targeting, a recent study showed that, in a mouse model of scleroderma, treatment with anti-BAFF antibody selectively diminished fibrosis-perpetuating IL-6, but not protective IL-10+ secreting B cells (Matsushita et al., 2018).

IL-10 production is important but it represents only one of the many mechanisms by which regulatory B cells suppress immune responses. Most studies of regulatory B cells have focused on autoimmune and inflammatory diseases and have identified various immunosuppressive strategies employed by these cells. These immunosuppressive functions include production of IL-35 and TGF-β, expansion of regulatory T cells, and generation of adenosine, an immunosuppressive metabolite (reviewed in Ray and Dittel, 2017). Other B cell—derived mechanisms that promote cutaneous tumor growth include IgG4 production, as well as induction of angiogenesis and lymphangiogenesis by B cells (Egbuniwe et al., 2015). Tumor-infiltrating B cells with elevated expression of TGF-β and PD-L1 were shown to curb activation and proliferation of CD8+ T cells (Liu et al., 2018). It will be important to further investigate the mechanisms by which regulatory B cells impede immunity and T cell responses to melanoma.

Of note, IL-10 has also been implicated in the protection against cancer, given its potent anti-inflammatory effects and the close association of chronic inflammation with the development of certain cancers (e.g., colon cancer) (Dennis et al., 2013). Under some circumstances, IL-10 even enhances effector functions in leukocytes, including cytotoxic T cells (Dennis et al., 2013). These findings further highlight the need to investigate IL-10—dependent immunosuppressive mechanisms for their ability to enhance growth of different skin cancers.

In summary, the work by Kobayashi et al. (2019) brings new concepts to our understanding of tumor immunity by demonstrating a crucial role for a subset of regulatory B cells directly within the tumor microenvironment. This work also illustrates that the specific depletion or inhibition of tumorigenic B cells, such as IL-10+ B cells, likely decreases melanoma growth, opening the door to novel approaches in the treatment of this deadly disease.

Clinical Implications
- Tumor-infiltrating regulatory B cells promote melanoma growth by producing IL-10.
- Intratumoral CD8+ T cell responses are dampened.
- Regulatory B cells and/or IL-10 represent potential therapeutic targets in melanoma.

**Figure 1.** IL-10+ regulatory B cells in the tumor microenvironment modulate anti-tumor immunity and tumor growth in melanoma. (a) When few IL-10+ regulatory B cells infiltrate melanoma, CD8+ T cell cytokine expression is unaffected, tumor immunity develops, and tumor growth is restrained. (b) When a large number of IL-10+ regulatory B cells is present in the tumor microenvironment of melanoma, IL-10 suppresses IFN-γ and TNF-α production by colocalizing CD8+ T cells, thereby attenuating tumor immunity. IL-10 also stimulates additional anti-tumor mechanisms, such as inhibition of innate immunity, induction of MDSCs, and downregulation of MHC class I and II antigen presentation in tumor cells and professional antigen presenting cells, respectively. Collectively, these mechanisms suppress tumor immunity and accelerate tumor growth. MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex.
Melanocyte homeostasis and their response to ultraviolet radiation (UVR) are mediated to a large extent by keratinocyte-derived factors, many of which have been well-characterized. Lee et al. describe novel effects of adenosine 5′-triphosphate (ATP), which is secreted by keratinocytes and can stimulate melanogenesis by melanocytes following UVA exposure. The investigators attribute the melanogenic effect of ATP to binding purinergic receptors type 2 X7 (P2X7), which are expressed on human melanocytes, leading to activation of the protein kinase C pathway. This report is the first to identify expression of specific purinergic receptors on human melanocytes, and it suggests ATP as a signaling molecule that stimulates pigmentation. Follow up on these results should clarify the physiological role of ATP in mediating the tanning response to solar UVR.


A symbiotic relationship exists between epidermal keratinocytes and melanocytes. Skin pigmentation is a process that involves the synthesis of melanin by epidermal melanocytes within specialized organelles, melanosomes, and transfer of fully melanized (mature) melanosomes to surrounding keratinocytes. Melanosomes localize in the perinuclear areas of keratinocytes and form supranuclear caps that limit penetration of solar UVR to nuclear DNA (Kobayashi et al., 1998). This confers photoprotection by reducing UVR-induced DNA damage and the risk of UVR signature mutations that are associated with skin cancers. In turn, keratinocytes regulate melanocyte homeostasis by producing paracrine factors that maintain melanocyte survival and regulate melanin synthesis (Imokawa, 2004; Swope and Abdel-Malek, 2018).

The production levels of many keratinocyte-derived paracrine factors are increased after exposure to UVR. Among these factors are endothelin-1 and α-melanocortin (i.e., α-MSH) that are known to have mitogenic and melanogenic effects on melanocytes (Swope and Abdel-Malek, 2018; Tada et al., 1998). These two factors promote repair of UVR-induced DNA photoproducts and inhibit UVR-induced apoptosis of melanocytes (Kadekar et al., 2010; von Koschmieder et al., 2015). These findings underscore the involvement of keratinocytes in reducing the genotoxic effects of UVR to maintain the genomic stability of melanocytes and to prevent malignant transformation. It is also known that aberrant production of paracrine factors is associated with pigmentary disorders, either hyperpigmentation (such as in melanoma or solar lentigines) or hypopigmentation (such as in vitiligo). Therefore, further elucidation of keratinocyte-melanocyte interactions is expected to lead to novel strategies aimed at modulating melanocyte function and ensuring normal pigmentation, as well as prevention of UVR-induced skin cancers.

Physiological significance of ATP and its effects on melanocytes

In this issue, Lee et al. (2019) describe a new mechanism by which keratinocytes can potentially stimulate the synthesis of melanin by melanocytes via the release of adenosine 5′-triphosphate (ATP) after UVA exposure. ATP is a