Cells to Surgery Quiz: October 2019

Oliver Taylor, BS¹ and Rajiv I. Nijhawan, MD¹


WHAT IS YOUR DIAGNOSIS?

Figure 1. Image courtesy of Rajiv Nijhawan, University of Texas Southwestern Medical Center.

Editorial note: Welcome to the Journal of Investigative Dermatology (JID) Cells to Surgery Quiz. In this monthly online-only quiz, the first question (“What is your diagnosis?”) relates to the clinical image shown, while additional questions concern the findings reported in the JID article by Kobayashi et al. (2019) (https://doi.org/10.1016/j.jid.2019.02.016).

Detailed answers and a list of relevant references are available following the Quiz Questions below.

QUIZ QUESTIONS

1. What is your diagnosis?
   a. Tinea Nigra
   b. Blue Nevus
   c. Solar Lentigo
   d. Melanoma
   e. Seborrheic Keratosis

¹Department of Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas

Correspondence: Rajiv I. Nijhawan, MD, University of Texas Southwestern Medical Center, Department of Dermatology, 5399 Harry Hines Blvd, Suite 400, Dallas, Texas 75390. E-mail: rajvnijhawan@gmail.com
2. What likely effect would increased PTEN have on B16F10 melanoma volume and mortality as described in Kobayashi et al. (2019)?
   a. PTEN expression increases B16F10 melanoma volume by increasing circulating IL-10–producing regulatory B cells (Bregs).
   b. PTEN expression increases B16F10 melanoma volume by increasing expression of phosphoinositide 3-kinase (P13K) in Bregs.
   c. PTEN expression decreases B16F10 melanoma volume by decreasing activation of CD8⁺ T cells.
   d. PTEN expression decreases B16F10 melanoma volume by increasing P13K activation in Bregs.
   e. PTEN expression decreases B16F10 melanoma volume by inhibiting protein kinase B activation in Bregs.

3. As described in Kobayashi et al. (2019), inhibition of what cell type specifically is a possible target for immunotherapy of melanomas?
   a. B1a Bregs
   b. CD20⁺ B cells
   c. CD8⁺ T cells
   d. CD4⁺ T cells
   e. T helper 17 cells

See following pages for detailed answers.
Melanoma is the deadliest cutaneous cancer (Siegel et al., 2019), and its incidence continues to rise. Melanomas are typically diagnosed through visual examination, with the goal being early detection. Clinicians utilize various approaches when determining whether a concerning lesion should be biopsied, including the assessment of pigmented lesions for one or more features that suggest melanoma, such as asymmetry; irregular borders; variegated color; diameter >6 mm; and a history of change in color, size, or shape (Abbasi et al., 2004). Suspicious lesions are also detected by the “ugly duckling sign,” which is when a suspicious lesion does not match the patient’s suspected nevus phenotype (Grob and Bonerandi, 1998). The supplemental use of dermoscopy, after some training, can also greatly improve the sensitivity and specificity of diagnosing melanomas (Neila and Soyer, 2011).

The most common distribution of lesion sites differs according to sex, the back for men and the arms and legs for women being the most common sites (Markovic et al., 2007). UV sunlight is the most important risk factor for the development of melanoma, with a history of sunburns in childhood conferring a higher risk than chronic continuous sun exposure (Rastrelli et al., 2014). The picture presented shows a superficial spreading melanoma (the most common invasive melanoma subtype) in the context of long-term sun exposure and elderly skin. Histologically, superficially spreading melanoma is characterized by a proliferation of atypical melanocytes along the dermoepidermal junction, which will often extend into the infundibular portion of hair follicles (Connolly et al., 2019). As the lesion transforms, poorly cohesive or dyshesive nests can be seen along the dermoepidermal junction aligned parallel to the long axis (known as the “swallow’s nest” sign) (Tannous et al., 2000). One can argue that the lesion in the figure falls under the category of a lentigo maligna melanoma (LMM) given the context of chronically sun-exposed skin. LMM is further distinguished by being located mainly on the head and neck.

Discussion of incorrect answers:

a. **Tinea Nigra:** Tinea nigra is a superficial mycosis caused by the fungus *Hortaea werneckii* and presents as an asymptomatic brown-black macule with irregular sharp margins (Nazzaro et al., 2016). Tinea nigra is rare in dry climates and the United States generally, and patients suspected of having tinea nigra should be asked about recent travel to tropical areas such as the Caribbean. Tinea nigra can be distinguished from melanoma most easily by scaling at the edge of the lesion and through dermoscopy that will often show brown spicules in a reticular-like pattern that does not follow the anatomy of the skin (Nazzaro et al., 2016). A more definitive diagnosis can be made by potassium hydroxide preparation, which will show numerous pigmented septate, variegated, and branched hyphae fungal elements (Bonifaz et al., 2008).

b. **Blue Nevus:** Also known as a blue mole, these are small blue-gray or blue-black papules that on histopathology show a collection of melanocytes found in the upper and mid-dermis (Cabral et al., 2014; Ojha et al., 2007). Blue nevi are benign acquired moles that often develop during young adulthood and have a higher incidence in women and those of Asian descent (Cabral et al., 2014). Blue nevi can be distinguished from melanoma by a lack of color variation, demoscopic patterns of globules, and a network (Baran and Duncan, 2011; Di Cesare et al., 2012). Definitive diagnosis can be made by biopsy for histologic evaluation.

c. **Solar Lentigo:** Also known as an age spot, liver spot, or senile lentigo, solar lentigo is a benign pigmented macule most commonly seen on fair-skinned individuals with a history of multiple sunburns. In those of Northern European descent, it is present in over 90% of individuals over 60 (Elgart, 2001). The appearance of the lesion is caused by an increase in basal melanin with a variable increase of epidermal melanocytes. It is important that solar lentigo be differentiated from lentigo maligna, also referred to as melanoma in situ, in the setting of sun-damaged skin. If a lentigo contains variegated pigmentation or changes, then a biopsy should be performed to rule out a malignancy (DeWane et al., 2019; Elgart, 2001). Solar lentigos can be limited through strategies to decrease UV exposure (sun avoidance, protective clothing, and sunscreen). Although these lesions are benign, solar lentigos can be treated through a variety of approaches including cryotherapy, laser, pulsed light, and chemical peels (Ortonne et al., 2006; Todd et al., 2000).

d. **Seborrheic Keratosis:** An extremely common benign neoplasm, seborrheic keratoses are often asymptomatic and increase in incidence and number with increasing age (Pariser, 1998). They often begin as a flat wrinkled plaque, with a classic stuck-on appearance. Plaques can grow to have a removable, coarse, waxy scale with a raw, moist base (Braun et al., 2017). They may become pruritic or painful, especially if located in an area that comes in close contact with clothing (bra strap being a typical
example). Rapid growth of many seborrheic keratoses is known as the sign of Leser-Trelat and is associated with visceral cancer (most commonly adenocarcinoma of the gastrointestinal tract) (Schwartz, 1996). Lesions are typically removed for cosmetic reasons or, more rarely, if lesions become irritated or inflamed. Removal is most commonly done through cryotherapy, although curettage and cautery has also been used (Herron et al., 2004).

2. What likely effect would increased PTEN have on B16F10 melanoma volume and mortality as described in Kobayashi et al. (2019)?

CORRECT ANSWER: e. PTEN expression decreases B16F10 melanoma volume by inhibiting protein kinase B activation in Bregs

Regulatory B cells (Bregs) inhibit inflammatory immune responses (Mizoguchi et al., 2000). In Bregs, phosphoinositide 3-kinase (PI3K) promotes IL-10 production by activating protein kinase B (Akt) (Matsushita et al., 2016). PTEN is an inhibitor of Akt activity; therefore, inactivating PTEN leads to increased Akt activity, which causes increased IL-10 production (Matsushita et al., 2016). IL-10 inhibitors are a known negative regulator of autoimmunity and inflammation (Bouaziz et al., 2008; Iwata et al., 2011; Matsushita et al., 2008).

Kobayashi et al. (2019) showed that in Bregs where PTEN was inactivated, there was a significant increase in B16F10 volume and decreased survival in PTEN-deficient mice injected subcutaneously with B16F10 melanoma cells compared with control mice (Kobayashi et al., 2019). See Figure 1 from Kobayashi et al. (2019).

Discussion of incorrect answers:

a. PTEN expression increases B16F10 melanoma cell volume by increasing circulating IL-10-producing Bregs. Although increasing IL-10 production would likely increase B16F10 melanoma, PTEN decreases IL-10 production in Bregs via an inhibition of Akt activity (Bouaziz et al., 2008; Iwata et al., 2011; Matsushita et al., 2016; Matsushita et al., 2008). The increased volume of B16F10 melanoma described resulted from an inactivation of PTEN (Kobayashi et al., 2019).

b. PTEN expression increases B16F10 melanoma cell volume by increasing expression of PI3K in Bregs. PI3K upregulates IL-10 in Bregs via activation of Akt, and upregulation of IL-10 in Bregs was shown to increase B16F10 melanoma volume in mice (Kobayashi et al., 2019). However, PTEN acts downstream of PI3K in Bregs to inhibit Akt activation, and therefore an increase in PTEN expression would likely decrease B16F10 melanoma volume rather than increase it (Kobayashi et al., 2019; Matsushita et al., 2016).

c. PTEN expression decreases B16F10 melanoma volume by decreasing activation of CD8 T cells. PTEN expression would likely decrease B16F10 melanoma volume via a decrease in IL-10 (Kobayashi et al., 2019). However, a decrease in IL-10 would increase the activation of CD8 T cells rather than decreasing CD8 activity (Bouaziz et al., 2008; Iwata et al., 2011; Matsushita et al., 2008).

d. PTEN expression decreases B16F10 melanoma volume by increasing PI3K activation in Bregs. As previously stated, PI3K upregulates IL-10 in Bregs via activation of Akt, and upregulation of IL-10 in Bregs was shown to increase B16F10 melanoma volume in mice (Kobayashi et al., 2019). PTEN acts downstream of PI3K in Bregs to inhibit Akt activation, and therefore an increase in PTEN expression would likely downregulate IL-10 production (Bouaziz et al., 2008; Iwata et al., 2011; Kobayashi et al., 2019; Matsushita et al., 2016; Matsushita et al., 2008).

3. As described in Kobayashi et al. (2019), inhibition of what cell type specifically is a possible target for immunotherapy of melanomas?

CORRECT ANSWER: a. B1a Bregs

Kobayashi et al. (2019) evaluated the role of B1a Bregs in relation to B16F10 melanoma growth in mouse models. When mice deficient in PTEN, an enzyme shown previously to expand Bregs when inactivated, were injected with B16F10 melanoma cells, their survival was decreased and volume of melanoma increased when compared with wild type mice (Matsushita et al., 2016). B1a Bregs injected into wild type mice, but not non-B1a B cells, significantly increased tumor growth and shortened animal survival when compared with the control. Adoptive transfer of B1a B cells from wild type mice enhanced B16F10 melanoma growth; however, adoptive transfer of B1a B cells from mice with a knockout of IL-10 showed no exacerbation of melanoma. Finally, in PTEN-deficient mice (in whom upregulation of B1a Bregs specifically had been shown previously), cytokine production by tumor-infiltrating CD8 T cells was found to be decreased compared with the control. This data suggests that B16F10 melanoma growth is enhanced by B1a Bregs.
specifically via an IL-10-mediated attenuation of CD8+ T cells. Accordingly, B1a Bregs may represent a possible target of for immunotherapy of melanoma.

**Discussion of incorrect answers:**

b. **CD20+ B cells.** B lymphocytes can both regulate cellular immune responses both positively and negatively. Kobayashi et al. (2019) demonstrated that CD19+, CD5+, CD43+, and B1a Bregs decrease immune response to B16F10. However, anti-CD20 antibodies-mediated depletion of B cells in mice enhanced B16 melanoma growth, with associated dysfunction of CD4+ and CD8+ T cells (DiLillo et al., 2010).

c. **CD8+ T cells.** IFN-γ production by CD8+ cells is an important mediator of antitumor immunity (Inoue et al., 2006). Kobayashi et al. (2019) demonstrated that inhibition of CD8+ cells via IL-10 enhances the growth of melanoma and therefore would be a poor target for melanoma immunotherapy.

d. **CD4+ T cells.** Cytokine production of CD4+ T cells is inhibited by IL-10 by downregulating IFN-γ, IL-4, and IL-5, making them poor targets for immunotherapy for melanoma (Del Prete et al., 1993). However, CD4+ T cells respond to B1a Bregs inhibition by downregulating IL-10 receptors, making mature CD4+ T cells mostly insensitive to IL-10 (Liu et al., 1994). This suggests that IL-10 inhibitory effects of antitumor immunity are most likely mediated by inhibited production of CD8+ T cells, rather than CD4+ T cells.

e. **T helper 17 cells.** T helper 17 cells are a subset of pro-inflammatory T helper cells, and were not studied in Kobayashi et al. (2019) (Hartigan-O’Connor et al., 2011).

REFERENCES


Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Ramagnani S. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 1993;150:353–60.


www.jidonline.org e119


