Cells to Surgery Quiz: March 2022

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**WHAT IS YOUR DIAGNOSIS?**

![Image](image_url)

*Figure 1.* Image courtesy of H. William Higgins II, Department of Dermatology, Hospital of the University of Pennsylvania.

*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 (*Quadri et al., 2022*).

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

**QUIZ QUESTIONS**

1. A man aged 81 years with a history of basal cell carcinoma and squamous cell carcinoma presents to the clinic with this lesion that has been increasing in size over the past 4 months. The patient is a retired teacher and currently lives by the beach. What is your diagnosis?
   a. Pigmented actinic keratosis
   b. Squamous cell carcinoma
   c. Sebaceous carcinoma
   d. Merkel cell carcinoma
   e. Melanoma

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2. **PA5 expression inhibited the EGF/EGFR signaling cascade, suggesting that protein kinase G activation leads to the inhibition of the EGFR pathway in melanoma.** What additional study results suggested EGF/EGFR inhibition?
   a. Increased phosphorylation of protein kinase B and extracellular signal–regulated kinase 1/2
   b. Decreased levels of cyclin B1 and increased levels of N-cadherin
   c. Decreased expression of BIM and decreased expression of survivin
   d. Increased levels of BIM and decreased levels of N-cadherin and cyclin B1
   e. A decreased percentage of fragmentation in melanoma spheroids

3. **An important result of the study was the antimetastatic effect of PA5 in vivo.** What results indicated this effect?
   a. The ability of PA5 to decrease cell viability in only WM115 and SKMEL-28 melanoma spheroids
   b. Increased fluorescence intensity of labeled injected cells of treated fish, suggesting cGMP inhibition on cell growth
   c. After treatment with PA5 (500 nM), only 10/20% of zebrafish showed initial metastases, and 78% of zebrafish had cells at the site of injection
   d. After PA5 treatment, phosphorylated VASP at serine 239 was detected in melanoma spheroids
   e. Treatment with PA5 (1,000 nM) reversed the metastatic trend, with only 78% of zebrafish showing cells in place.

See following pages for detailed answers.
DETAILED ANSWERS

1. A man aged 81 years with a history of basal cell carcinoma and squamous cell carcinoma presents to the clinic with this lesion that has been increasing in size over the past 4 months. The patient is a retired teacher and currently lives by the beach. What is your diagnosis?

CORRECT ANSWER: e. Melanoma.

The diagnosis is melanoma. Melanoma classically presents as an evolving macule or nodule with an asymmetric border and color variegation. These features are often described as the ABCDEs of melanoma. The lesion in the photograph has the characteristic ABCD criteria, including asymmetry (i.e., A), an irregular border (i.e., B), variation in color (i.e., C), and a diameter ≥6 mm (i.e., D). E denotes evolution over time, which cannot be discerned from the photograph but was described in the question stem. Melanomas are often classified by their growth pattern, which may affect the clinical presentation of the lesion (Kibbi et al., 2016). Subtypes include superficial spreading, nodular, lentigo maligna, and acral lentiginous melanoma.

Discussion of incorrect answers:

a. Pigmented actinic keratosis: Actinic keratosis presents as an erythematous papule with an adherent scale or palpable rough surface on chronically sun-exposed skin. Most lesions lack color, but pigmentation can occur. If present, lesions may appear as irregularly pigmented, poorly defined macules or patches with foci of keratotic scale (Chung et al., 2013). This presentation can mimic diagnoses such as melanoma. The lesion in the photograph does not have a keratotic scale and has the characteristic ABCDE features of melanoma.

b. Squamous cell carcinoma: Squamous cell carcinoma (SCC) presents with scaling, ulceration, or a cutaneous horn. SCC typically has a hyperkeratotic appearance, in contrast to the tumor in the photograph.

c. Sebaceous carcinoma: The presentation of sebaceous carcinoma is heterogeneous. Sebaceous carcinoma may appear as an umbilicated papule, ulcerated lesion, or subcutaneous nodule with exophytic growth. Lesions may be clinically mistaken for tumors such as basal cell carcinoma or SCC.

d. Merkel cell carcinoma: Merkel cell carcinoma (MCC) is an aggressive cutaneous neuroendocrine malignancy that presents as a rapidly evolving, flesh-colored, or violaceous nodule. MCC commonly appears on sun-exposed skin in elderly patients.

2. PA5 expression inhibited the EGF/EGFR signaling cascade, suggesting that protein kinase G activation leads to the inhibition of the EGFR pathway in melanoma. What additional study results suggested EGF/EGFR inhibition??

CORRECT ANSWER: D. Increased levels of BIM and decreased levels of N-cadherin and cyclin B1.

The EGF/EGFR signaling cascade is a pathway that regulates important cellular processes such as growth, survival, and proliferation. Overexpression of this pathway has been shown to correlate with malignancy metastases, chemotherapy resistance, and poor prognostic outcomes (Lemmon and Schlessinger, 2010). EGF/EGFR signaling increases the levels of cellular proteins, including cyclin B1 and N-cadherin. Cyclin B1 plays an important regulatory role in cell cycle progression (Castedo et al., 2002), whereas N-cadherin mediates melanoma cell invasion (Ciołczyk-Wierzbicka and Laidler, 2018). Conversely, EGF/EGFR signaling decreases the production of pro-apoptotic proteins such as BIM.

In the study, the authors showed that PA5 expression can lead to the inhibition of the EGF/EGFR signaling cascade in melanoma. By inhibiting EGF/EGFR signaling and downstream transducers, results ultimately showed decreased levels of N-cadherin and cyclin B1 and increased levels of BIM.

Discussion of incorrect answers:

a. Increased phosphorylation of protein kinase B and extracellular signal-regulated kinase 1/2: The EGF/EGFR pathway has several important transducers, including protein kinase B (Akt) and extracellular signal–regulated kinase (ERK) 1/2. The pathway is initially activated through EGFR phosphorylation, leading to downstream EGF signaling. EGF signaling promotes the activation and phosphorylation of Akt and ERK1/2.

In the study, PA5 blocked EGFR phosphorylation in the SKMEL-28 cell line and M121224 metastatic cells. This led to decreased phosphorylation of Akt and ERK1/2 and inhibition of downstream signaling.

b. Decreased levels of cyclin B1 and increased levels of N-cadherin: Cyclin B1 and N-cadherin levels
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increase in response to EGF/EGFR signaling. In response to PA5 expression and EGF/EGFR inhibition, both cyclin B1 and N-cadherin would decrease.

c. **Decreased expression of BIM and decreased expression of survivin:** BIM is a pro-apoptotic, BCL2-family protein involved in the intrinsic apoptotic pathway. Survivin is a protein encoded by BIRC5 mRNA involved in apoptosis inhibition. In response to PA5 expression, BIM levels significantly increased, and survivin expression decreased. Together, these results confirmed the ability of PA5 to reduce melanoma spheroid cell invasion, growth, and survival, not EGF/EGFR signaling.

e. **A decreased percentage of fragmentation in melanoma spheroids:** The percentage of fragmentation represents an individual or clustered cells released from melanoma spheroids. The percentage of fragmentation assessed whether PA5 affected the invasive capacity of melanoma cells, not EGF/EGFR signaling. The percentage of fragmentation was significantly lower in PA5-treated spheroids than in controls.

3. An important result of the study was the antimetastatic effect of PA5 in vivo. What results indicated this effect?

**CORRECT ANSWER:** e. Treatment with PA5 (1,000 nM) reversed the metastatic trend, with only 78% of zebrafish showing cells in place.

To assess the efficacy of PA5, metastatic melanoma cells were injected into zebrafish larvae. Zebrafish were subsequently injected with scaling doses of PA5 (0, 500, 1,000 nM) and monitored for 4 days. PA5 (1,000 nM) treatment completely reversed the metastatic trend, with 78% of zebrafish showing cells solely at the site of injection (cells in place). A reduction in melanoma cell mass was also observed as well as reduced fluorescence intensity compared with that of the controls. Treatment with PA5 (1,000 nM) differed from that with PA5 (500 nM), where approximately 40/50% of zebrafish showed initial metastasis, and 50/60% of zebrafish had cells at the site of injection.

**Discussion of incorrect answers:**

a. **The ability of PA5 to decrease cell viability in only WM115 and SKMEL-28 melanoma spheroids:** The authors showed PA5’s ability to reduce cell viability in all melanoma spheroids with scaling doses (100, 250, 500, 1,000 nM), whereas PA4 was only effective on WM115 and SKMEL-28 spheroids. PA5 (250 nM) was also efficacious in human metastatic spheroids 24 hours after treatment.

b. **Increased fluorescence intensity of labeled injected cells of treated fish, suggesting cGMP inhibition on cell growth:** M121224 and M130425 metastatic melanoma cells were stained and injected into zebrafish larvae. The fluorescence intensity was measured to quantify melanoma cell mass. After treatment with PA5, fluorescence intensity was significantly reduced in treated fish compared with that in the controls. Decreased fluorescence intensity suggested cGMP inhibition on cell growth.

c. **After treatment with PA5 (500 nM), only 10/20% of zebrafish showed initial metastases, and 78% of zebrafish had cells at the site of injection:** PA5 treatment (500 nM) did decrease metastases, with 40/50% of zebrafish showing only initial metastases and 50/60% of zebrafish with cells at the initial site of injection.

d. **After PA5 treatment, phosphorylated VASP at serine 239 was detected in melanoma spheroids:** Protein kinase G (PKG) is a downstream transducer of the cGMP signaling pathway. PA5 is a cGMP analog that activates PKG. VASP is a downstream target of PKG, and PKGs preferentially phosphorylate VASP at serine 239. After PA5 treatment and thus PKG amplification, phosphorylated VASP at serine 239 was detected. This result did not indicate an antimetastatic effect but rather confirmed the activation of PKGs by PA5.

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**CONFLICT OF INTEREST**

The authors state no conflict of interest.

**REFERENCES**


