Cells to Surgery Quiz: November 2019

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WHAT IS YOUR DIAGNOSIS?

Figure 1. Image courtesy of Joseph F. Sobanko MD, University of Pennsylvania Department of Dermatology.

Editorial note: Welcome to the Journal of Investigative Dermatology (JID) Cells to Surgery Quiz. In this monthly online-only quiz, the first question relates to the clinical image shown, while additional questions concern the findings reported in the JID article by Atkinson et al. (2019) (https://doi.org/10.1016/j.jid.2018.10.038).

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

QUIZ QUESTIONS

1. This is a photo of a 30-year-old patient with an invasive squamous cell carcinoma that appeared initially as a nonhealing wound on the foot. What is the gene defect in this patient’s underlying condition?
   
   a. XPA
   b. MSH1/MSH2
   c. TYR
   d. COL7A1
   e. PTCH1

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2. **Ectopic** \( P4HA3 \) **gene expression increases basal proliferation rates and increases invasiveness of PMWK cells. Which of the following is true about methylation status of** \( P4HA3 \) **in melanomas?**

   a. The CpG islands of the gene are largely unmethylated in malignant melanocytes.
   
   b. The density of gene methylation in melanoma is significantly lower than that of benign melanocytic nevi, and even less methylation is observed in some nodal metastatic melanoma lesions.
   
   c. The density of gene methylation in melanoma is significantly lower than that of benign melanocytic nevi, but a greater density of methylation is observed in some nodal metastatic melanoma lesions.
   
   d. The density of gene methylation in melanoma is significantly higher than that of benign melanocytic nevi, but a lower density of methylation is observed in some nodal metastatic lesions.
   
   e. The density of gene methylation in melanoma is significantly higher than that of benign melanocytic nevi, and an even greater density of methylation is observed in some nodal metastatic lesions.

3. **The tumor suppressor function of C-3PH/LEPREL1** was confirmed by which of the following results in validation studies?

   a. Inhibited cell growth in melanoma cell lines with ectopic LEPREL1 overexpression
   
   b. Significantly lower methylation of LEPREL1 in melanoma compared with benign nevi
   
   c. Significantly worse disease-free survival for melanoma cases with overexpression of C-3PH
   
   d. Loss of malignant potential in melanoma cell lines by inhibition of LEPREL1 with ethyl-3,4-dihydroxybenzoate
   
   e. Downregulation of LEPREL1 gene expression in melanoma cell lines treated with demethylation agents 5' azacytidine and trichostatin A

See following pages for detailed answers.
1. This is a photo of a 30-year-old patient with an invasive squamous cell carcinoma that appeared initially as a nonhealing wound on the foot. What is the gene defect in this patient's underlying condition?

**CORRECT ANSWER: d. COL7A1**

This patient with skin erosion and digit amputation has dystrophic epidermolysis bullosa (DEB), an inherited blistering disorder caused by a mutation in the COL7A1 gene. COL7A1 is a protein-coding gene responsible for type VII collagen formation. Type VII collagen is the main structural constituent of anchoring fibrils, which are located in the basement membrane and attach the epidermis to the dermis. Mutations in this gene lead to a defective function or reduced number of fibrils, thus disrupting the integrity of the dermo-epidermal junction. The severity of disease presentation is dependent on the type of mutation. Recessive DEB-Hallopeau-Siemens subtype is the most severe phenotype, caused by premature termination codon mutations and a consequent lack of anchoring fibrils. Less severe phenotypes include recessive DEB—non-Hallopeau-Siemens subtype, caused by missense or frameshift COL7A1 mutations, and dominant DEB, caused by glycine substitutions that interfere with the triple helix assembly of type VII collagen (Spitz and Hatch, 2005).

Recessive DEB presents with generalized bullae, pigmentation changes, mucosal erosions, and nail dystrophy and loss, as well as possible musculoskeletal and hematologic manifestations (Spitz and Hatch, 2005). Patients may also have mitten deformities because of excessive scarring of the hands and feet (Bruckner-Tuderman, 2010). A clinical hallmark is trauma-induced skin blisters that heal with atrophic scarring; these chronic wounds are at increased risk for developing squamous cell carcinoma (SCC), with a lifetime risk of over 90% (Pfendner and Lucky, 1993). The risk of developing SCC, a major cause of premature death in this population, is correlated with blistering severity (Fine et al., 1999). Recurrent skin cancer may lead to amputation, as seen in this patient. Patients with epidermolysis bullosa require management with a multidisciplinary team involving a dermatologist, nutritionist, ophthalmologist, orthopedic surgeon, and psychiatrist, depending on disease severity. Goals of care include infection prevention, nutrition support, skin cancer surveillance, and genetic counseling (Spitz and Hatch, 2005).

**Discussion of incorrect answers:**

a. **XPA**: Genetic defects in XPA are associated with xeroderma pigmentosum (XP), a rare, genetically heterogeneous disorder associated with defects in DNA repair enzymes. XP is characterized into eight different complements, each of which is associated with a gene mutation that codes for a different protein involved in nucleotide excision repair (NER) (Spitz and Hatch, 2005). NER is a form of DNA repair that is critical for the repair of UV-induced DNA damage; consequently, patients with XP are particularly susceptible to UV exposure—associated cutaneous cancers at a young age. Compared with the general population, patients with XP have a 10,000-fold increased lifetime risk of nonmelanocytic skin cancer (basal cell carcinoma [BCC] or squamous cell carcinoma) and a 2,000-fold increased risk of melanoma (Black, 2016). XP presents early in childhood with primarily cutaneous symptoms, including severe sun sensitivity and early development of poikiloderma. Patients with XP are also susceptible to UV-induced ocular disease, and 20% of patients experience neurologic symptoms that are considered to be unrelated to the degree of UV exposure. Management involves strict avoidance of UV light as well as frequent skin cancer screening for early detection of malignancy, adherence to which significantly prolongs survival (Spitz and Hatch, 2005).

b. **MSH1/MSH2**: MSH1 and MSH2 are the two genes mutated in Muir-Torre Syndrome (MTS), a subset of hereditary nonpolyposis colorectal carcinoma syndrome. MSH1 and MSH2 encode proteins responsible for DNA mismatch repair, an integral system that detects erroneous nucleotide insertions, deletions, or changes in the DNA of all cells. Mutations in the mismatch repair system lead to the persistence of undetected errors in DNA and contribute to areas of microsatellite instability responsible for an increased risk of both visceral and cutaneous neoplasms. MTS usually presents in the fifth or sixth decade of life, and indolent internal malignancies such as adenocarcinomas of the colon are often discovered first. The skin findings in MTS include multiple sebaceous tumors, most commonly adenomas. These benign tumors appear as tan or pink nodules or papules usually less than 5 mm in diameter, concentrated on the head and neck (Shalin et al., 2010). BCCs with sebaceous differentiation and keratoacanthomas with typical flesh-colored, umbilicated lesions can also be seen (Spitz and Hatch, 2005).

c. **TYR**: Mutations in TYR are associated with oculocutaneous albinism type 1 (OCA1). OCA1 is an autosomal recessive form of albinism characterized by absence of the tyrosinase enzyme, encoded by the gene TYR. Tyrosinase is an essential enzyme in
the biosynthesis of melanin. Patients with OCA1 have a normal number of melanocyte cells but demonstrate a complete lack of pigment in their skin, hair, and eyes (Kamaraj and Purohit, 2014). Other forms of albinism are also characterized by decreased pigment, though they are considered less severe than OCA1 as some melanin accumulates in the tissues over time. Clinically, patients with this disorder present with snow-white hair, pink-white skin, and significant ocular symptoms including decreased visual acuity, nystagmus, strabismus, and photophobia. Patients have an increased risk of skin cancer, particularly squamous cell carcinoma, although BCC and melanoma also occur. Management of patients with OCA1, as with most types of albinism, includes avoidance of UV exposure, regular skin cancer screening, and ophthalmologic care (Spitz and Hatch, 2005).

e. **PTCH1**: Basal cell nevus syndrome is an inherited autosomal dominant syndrome caused by mutations in **PTCH1**. **PTCH1** encodes a protein by the same name that functions to inhibit the transmembrane regulator protein SMO. Loss of **PTCH1**-dependent inhibition of SMO leads to unregulated intracellular signaling in the Sonic Hedgehog pathway, contributing to abnormal cell proliferation and the generation of numerous BCCs (Lam et al., 2013). BCC formation begins in childhood in patients with basal cell nevus syndrome. The BCCs that develop in this condition can differ in appearance from the typical flesh-colored plaques seen in adults. They are often small, brown, dome-shaped papules and primarily found on the face, back, or chest (Spitz and Hatch, 2005).

2. **Ectopic P4HA3 gene expression increases basal proliferation rates and increases invasiveness of PMWK cells.** Which of the following is true about methylation status of P4HA3 in melanomas?

**CORRECT ANSWER: e. The density of gene methylation in melanoma is significantly higher than that of benign nevi, and an even greater density of methylation is observed in some nodal metastatic lesions.**

**P4HA3** encodes one of the alpha catalytic subunits of the tetrameric protein C-P4H. C-P4H is a protein necessary for the post-translational processing of collagen in cells. Little is known about the functions of P4HA3, but members of its gene family such as P4HA1 and P4HA2 have a known role in breast cancer metastasis. At face value, the methylation data for **P4HA3** suggests that the gene has a tumor suppressor function. In an assessment of 50 melanoma cases with benign pigmented nevi as controls, the level of methylation for **P4HA3** was significantly higher in melanoma than in benign nevi (P < 0.001). Furthermore, for malignant melanomas, the density of **P4HA3** methylation is higher in some lymph node metastases than primary cases (P < 0.01). Despite this observation, when PMWK cells are transfected with **P4HA3** plasmids, a significant increase in basal proliferation rate and invasiveness is observed, indicating that **P4HA3** has an oncogenic function. Methylation-dependent transcriptional silencing of a gene with potential oncogenic function is counterintuitive, but has been described before with **NT5E** in both breast cancer and metastatic melanoma. In these cancers, better clinical outcomes are seen in cases with methylation of **NT5E**. A similar phenomenon may be observed with **P4HA3** gene silencing in the future as this article includes observational data that disease-free survival for melanoma is significantly worse in cases overexpressing **P4HA3** (P = 0.0064). These findings suggest that C-P4H inhibition reduces phenotypic aggression by inhibiting invasiveness and angiogenesis, and thus C-P4H may be a valuable therapeutic target for reducing invasion in melanoma.

**Discussion of incorrect answers:**

a. **The CpG islands of the gene are largely unmethylated in malignant melanocytes.** mRNA expression of **P4HA3** was undetectable in SBCL2, SKMEL23, SKMEL30, SKMEL505, and C8161 melanoma cell lines and detectable at very low levels in SKMEL147, SKMEL224, and WM266.4 melanoma cell lines. Correlation analysis demonstrates that expression of **P4HA3** was negatively associated with methylation (r = –0.5496, P = 0.0417) implying that CpG island methylation of **P4HA3** is present in malignant melanocytes. However, the CpG islands of **P4HA1** and **P4HA2** genes, known to be important in cancer metastasis, were generally unmethylated.

b. **The density of gene methylation in melanoma is significantly lower than that of benign melanocytic nevi, and even less methylation is observed in some nodal metastatic melanoma lesions.**

c. **The density of gene methylation in melanoma is significantly lower than that of benign melanocytic nevi, but a greater density of methylation is observed in some nodal metastatic melanoma lesions.**

Answer choices b and c are incorrect because the authors found that for the gene **P4HA3**, levels of methylation were significantly higher in melanoma than in benign nevi (P < 0.001) (Supplementary Figure 3b).

d. **The density of gene methylation in melanoma is significantly higher than that of benign melanocytic nevi, but a lower density of methylation is observed in some nodal metastatic lesions.**
The first part of this answer choice is true, P4HA3 density of methylation is significantly higher in melanoma than in benign melanocytic nevi ($P < 0.001$) (Supplementary Figure 3b). However, the second part is false. When density of P4HA3 methylation was assessed in metastatic melanoma, some nodal lesions had significantly higher levels of methylation than primary lesions ($P < 0.01$).

3. The tumor suppressor function of C-3PH/LEPREL1 was confirmed by which of the following results in validation studies?

CORRECT ANSWER: a. Inhibited cell growth in melanoma cell lines with ectopic LEPREL1 overexpression

The authors suspected that C-3PH/LEPREL1 and LEPREL2 have a tumor-suppressor function based on the high level of C-3PH methylation and low level of LEPREL1 and LEPREL2 mRNA expression in melanoma compared with benign nevi. To validate this finding, they transfected the melanoma cell line SKMEL501, which has methylated LEPREL1 and LEPREL2, with an expression plasmid that overexpressed C-3PH. They found that ectopic expression of LEPREL1 and LEPREL2 completely inhibited cell proliferation in SKMEL501 cells.

LEPREL1 is a prolyl-3-hydroxylase that is partially responsible for the post-translational processing of collagen. Specifically, it is involved in processing type IV collagen, an important component of the basement membrane and a subject of interest given that compromise of the basement membrane is a common feature of cancer cells. The authors have previously demonstrated that C-3PH genes are tumor suppressors that are transcriptionally silenced in breast cancer.

Discussion of incorrect answers:

b. Significantly lower methylation of LEPREL1 in melanoma compared with benign nevi: Methylation of LEPREL1 was significantly greater in melanoma than in benign nevi ($P \leq 0.0001$) in a series of 50 clinical melanoma cases. The significance of this was further investigated by the authors through the use of pyrosequencing to test for methylation of C-3PH in a number of different melanoma and melanocyte cell lines. They found that C-3PH was unmethylated in normal melanocytes but was methylated in a number of melanoma cell lines. In those cell lines, methylation was negatively correlated ($r = -0.3573$) with LEPREL1 gene expression; in three of the cell lines where C-3PH was densely methylated (SKMEL2, SKMEL23, and SKMEL501), LEPREL1 gene expression was undetectable.

c. Significantly worse disease-free survival for melanoma cases with overexpression of C-3PH: This is true for P4HA2 and P4HA3; the authors did not investigate the relationship between LEPREL1 expression and disease-free melanoma survival.

d. Loss of malignant potential in melanoma cell lines by inhibition of LEPREL1 with ethyl-3,4-dihydroxybenzoate: Melanoma cell lines PMWK and SKMEL23 treated with ethyl-3,4-dihydroxybenzoate (EDHB) did demonstrate antiproliferative activity; however, LEPREL1 is not the substrate of EDHB. The authors determined that the mechanism of EDHB is induction of apoptosis through the targeting of P4HA2 and P4HA3.

e. Downregulation of LEPREL1 gene expression in melanoma cell lines treated with demethylation agents 5′ azacytidine and trichostatin A: Demethylation studies of SKMEL501, a melanoma cell line that does not express endogenous LEPREL1 or LEPREL2 because of methylation, demonstrated an increase in LEPREL1 expression via quantitative PCR after treatment with 5′ azacytidine and trichostatin A compared with untreated SKMEL501 cells. Therefore, LEPREL1 gene expression is upregulated when demethylated.

ACKNOWLEDGMENTS

Jeremy R. Etzkorn is supported by a Career Development Award in Dermatologic Surgery from the Dermatology Foundation.

REFERENCES


