Cells to Surgery Quiz: September 2019

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WHAT IS YOUR DIAGNOSIS?

Figure 1. Image courtesy of Eva A. Hurst, MD.

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 Kuonen et al. (2019) (https://doi.org/10.1016/j.jid.2018.11.035).

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

QUIZ QUESTIONS

1. What is your diagnosis?
   a. Squamous cell carcinoma of the skin
   b. Sebaceous hyperplasia
   c. Trichoepithelioma
   d. Basal cell carcinoma in basal cell nevus (Gorlin) syndrome
   e. Merkel cell carcinoma

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2. What is true about the development and progression of squamous cell carcinomas?
   a. Squamous cell carcinomas depend on activation of Ras/MAPK signaling and loss of Notch signaling
   b. Squamous cell carcinomas depend on loss of Ras/MAPK signaling and activation of Hedgehog signaling
   c. Squamous cell carcinomas depend on activation of Ras/MAPK signaling and activation of Notch signaling
   d. Squamous cell carcinomas depend on loss of Ras/MAPK signaling and loss of Notch signaling
   e. Squamous cell carcinomas depend on loss of Ras/MAPK signaling and loss of Hedgehog signaling

3. Kuonen et al. (2019) presented their findings on the loss of primary cilia in driving pathway switching in Smo inhibitor-resistant basal cell carcinomas. Which of the following is a conclusion of their research?
   a. There are more mutations in primary ciliary genes in Gorlin syndrome basal cell carcinomas than sporadic naive basal cell carcinomas, followed by Smo inhibitor-resistant basal cell carcinomas
   b. Loss of primary cilia correlates with Hedgehog pathway activation
   c. Ras/MAPK activation occurs in treatment-resistant basal cell carcinomas that express high levels of Hedgehog pathway genes
   d. Loss of primary cilia potentiates Ras/MAPK activation
   e. Ras/MAPK pathway activation confers resistance to MEK inhibitors in Smo inhibitor-resistant basal cell carcinomas

See following pages for detailed answers.
DETAILED ANSWERS

1. What is your diagnosis?

CORRECT ANSWER: d. Basal cell carcinoma in basal cell nevus (Gorlin) syndrome

Basal cell carcinomas are typically described as shiny, pearly, pink to brown papules or plaques with rolled borders and telangiectasias, more commonly found on sun-exposed areas. Some can be ulcerated or pigmented. Gorlin syndrome, or basal cell nevus syndrome, is an inherited autosomal dominant condition with mutations in PTCH1, a tumor suppressor gene, that predisposes the individual to developing multiple basal cell carcinomas of the skin (Hahn et al., 1996). Patients with Gorlin syndrome are also at risk for other systemic disorders, such as odontogenic tumors, skeletal abnormalities, ovarian fibromas, and so forth.

PTCH1 is a transmembrane protein that destabilizes Smoothened (Smo), but on binding of Hedgehog to Patched, Smo is no longer inhibited and can activate the downstream transcription factor GLI to initiate cell proliferation. With deactivating mutations in PTCH1, such as seen in Gorlin syndrome, it can lead to unregulated activation of the Hedgehog pathway and lead to malignancies such as basal cell carcinomas (BCCs).

Other physical features seen in patients with Gorlin syndrome are small pits on the palms and soles, mandibular odontogenic cysts, pectus excavatum, scoliosis, and kyphosis. Characteristic facies include hypertelorism, frontal bossing, and broad nasal root. The diagnosis of Gorlin syndrome is based on clinical findings and is based on fulfilling either two major criteria or one major and two minor criteria (Evans and Farndon, 1993):

<table>
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<tr>
<th>Major criteria</th>
<th>Minor criteria</th>
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<tr>
<td>More than 2 BCCs or 1 BCC under 20 years</td>
<td>Childhood medulloblastoma</td>
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<tr>
<td>Odontogenic keratocyst (histology proven)</td>
<td>Congenital malformations (cleft lip/palate, frontal bossing, coarse face, moderate/severe hypertelorism)</td>
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<tr>
<td>3 or more palmar or plantar pits</td>
<td>Other skeletal abnormalities (Sprengel deformity, marked pectus deformity, marked syndactyly of digits)</td>
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<td>Lamellar calcification of the falx cerebri or clear evidence of calcification younger than 20 years</td>
<td>Radiologic abnormalities (bridging of the sella turcica, vertebral anomalies, modeling defects of hands and feet, flame-shaped luencies of hands or feet)</td>
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<td>Bifid, fused or markedly splayed ribs</td>
<td>Ovarian or cardiac fibroma</td>
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<tr>
<td>First-degree relative with Gorlin syndrome</td>
<td>PTCH mutation</td>
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<tr>
<td>Eye anomaly (cataract, coloboma, microphthalmia)</td>
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The clinical photographs show multiple BCCs that can be seen in a patient with Gorlin syndrome.

Discussion of incorrect answers:

a. Squamous cell carcinoma of the skin: like BCCs, cutaneous squamous cell carcinomas (SCCs) often occur on sun-exposed sites, because UV exposure from the sun can cause DNA damage and malignant transformation. SCCs are typically scaly red papules and plaques that may ulcerate and are commonly associated with mutations in p53, Notch, and Ras pathways. Patients with Gorlin syndrome are not predisposed to developing squamous cell carcinoma skin cancers. There is some evidence associating overactivation of the Hedgehog pathway and cutaneous SCCs (Ping et al., 2001); however, no mechanistic relationship has been established.

b. Sebaceous hyperplasias: are benign, small yellow papules up to 3–5 mm in diameter found commonly on the forehead and cheeks. They often have a central dimple with prominent blood vessels, and can sometimes mimic BCCs. Mutations in the Ras pathway (KRAS, HRAS) and EGFR have been discovered in sebaceous hyperplasias (Groesser et al., 2016).

c. Trichoepitheliomas: are benign but uncommon tumors that can arise from hair follicles and arise on the face (cheeks, eyelids, and nose) after puberty. They are typically small, firm, round, shiny, and can be yellow, pink, brown, or blue. They can be confused with basal cell carcinomas, and can occasionally develop into BCCs. People with Brooke-Spiegler syndrome, an inherited autosomal dominant
condition with mutations in the CYLD protein, are predisposed to developing multiple trichoepitheliomas, as well as cylindromas and spiradenomas (Bignell et al., 2000). PTCH mutations have also been found in benign trichoepitheliomas, and, notably in these cases, the mutation was not sufficient for tumorigenesis (Vorechovský et al., 1997).

e. **Merkel cell carcinoma:** is a rare skin cancer that is typically a solitary red nodule most often on the head and neck of elderly or immunosuppressed individuals. They are aggressive, rapidly growing, and have a high metastatic potential and high rates of recurrence. The Merkel cell polyomavirus (MCV) is detected in 75–80% of all Merkel cell carcinomas and is considered pathogenic in those cases (Feng et al., 2008). The remaining cases of MCV-uninfected Merkel cell carcinoma have an unknown cause but are typically associated with UV exposure and high genome mutation rates (Schrama et al., 2012).

2. What is true about the development and progression of squamous cell carcinomas?

**CORRECT ANSWER:** a. Squamous cell carcinomas depend on activation of Ras/MAPK signaling and loss of Notch signaling

Squamous cell carcinomas (SCCs) can arise during Smo inhibition with vismodegib for the treatment of BCCs. The switching of BCCs to SCC is proposed to be a potential route to developing resistance to vismodegib (Ransohoff et al., 2015; Zhao et al., 2015). SCCs depend on activation of the the Ras/MAPK pathway for proliferation, and loss of Notch signaling for blocking of differentiation (Lefort et al., 2007; Li et al., 2015). Notch signaling is under p53 tumor suppressor control, and p53 promotes Notch signaling to drive differentiation and growth suppression. Loss of p53 and/or loss of Notch signaling blocks differentiation and promotes tumor promotion (Yugawa et al., 2007). SCCs are not dependent on the Hedgehog signaling pathway for cell proliferation, whereas BCCs do depend on the activation of Hedgehog signaling.

**Discussion of incorrect answers:**

b. **Squamous cell carcinomas depend on loss of Ras/MAPK signaling and activation of Hedgehog signaling:** SCCs depend on the activation of Ras/MAPK signaling to promote proliferation. This choice of answer is incorrect because the loss of Ras/MAPK signaling would inhibit proliferation. Furthermore, SCCs do not depend on the activation of Hedgehog signaling. Some SCCs have been shown to carry mutations in PTCH, but it is still unclear whether these mutations are driver or passenger mutations (Ping et al., 2001).

c. **Squamous cell carcinomas depend on activation of Ras/MAPK signaling and activation of Notch signaling:** SCCs depend on the activation of Ras/MAPK signaling to promote proliferation. However, this choice of answer is incorrect because SCCs do indeed depend on the loss of Notch signaling for blocking of differentiation (Lefort et al., 2007). The activation of Notch signaling would promote differentiation and inhibit the progression of SCCs.

d. **Squamous cell carcinomas depend on loss of Ras/MAPK signaling and loss of Notch signaling:** SCCs depend on the activation of Ras/MAPK signaling to promote proliferation. This choice of answer is incorrect because the loss of Ras/MAPK signaling would inhibit proliferation. However, SCCs do indeed depend on the loss of Notch signaling.

e. **Squamous cell carcinomas depend on loss of Ras/MAPK signaling and loss of Hedgehog signaling:** SCCs depend on the activation of Ras/MAPK signaling to promote proliferation. This choice of answer is incorrect because loss of primary cilia potentiated Ras/MAPK signaling.

3. **Kuonen et al. (2019) presented their findings on the loss of primary cilia in driving pathway switching in Smo inhibitor-resistant basal cell carcinomas. Which of the following is a conclusion of their research?**

**CORRECT ANSWER:** d. Loss of primary cilia potentiates Ras/MAPK activation

Inhibition of primary cilia formation with ciliobrevin D in ASZ cells (a GLI-dependent murine BCC cell line) or UWBCC cells (a GLI-dependent human BCC line) did not enrich for Ras pathway genes, indicating that loss of cilia was not sufficient to activate the Ras pathway. However, using an inducible Ras activation system, the authors showed that loss of primary cilia was able to potentiate Ras signaling. Furthermore, this result was replicated when the inhibition of primary cilia was achieved using a small interfering RNA to OFD1 (essential for cilia formation). The authors propose that resistance correlates with loss of the primary cilium, which acts as a gatekeeper to prevent switching of Hedgehog to Ras/MAPK signaling. Recently, another group has also identified reduction in Hedgehog and activation of Ras/MAPK upon loss of primary cilia switching in resistant medulloblastoma.
(Zhao et al., 2015). The authors suggest therapies to target the crosstalk between primary cilia and Ras/MAPK signaling to potentially reduce resistance in the treatment of BCCs.

**Discussion of incorrect answers:**

- **a. There are more mutations in primary ciliary genes in Gorlin syndrome basal cell carcinomas than sporadic naive basal cell carcinomas:** Kuonen et al. (2019) found significantly higher numbers of ciliary mutations in vismodegib-resistant BCCs compared with sporadic naive BCCs, followed by Gorlin syndrome BCCs. The high mutational load resulted in fewer numbers of cilia in treatment-resistant BCCs. Interestingly, patients with Gorlin syndrome have shown significantly less resistance to vismodegib (Tang et al., 2012), and Gorlin syndrome BCCs treated with vismodegib had conserved numbers of cilia compared with treatment-resistant BCCs. The authors then go on to show that primary cilia are important in the prevention of treatment resistance as it inhibits the switching from Hedgehog to Ras/MAPK pathways.

- **b. Loss of primary cilia correlates with Hedgehog pathway activation:** The authors quantified primary cilia, nuclear Gli1 (downstream of Hedgehog pathway activation), and phosphorylated (p-)MEK (downstream of Ras/MAPK pathway activation) with immunostaining on treatment-resistant BCCs. They found that cilia were conserved in Gli1/high/p-MEK/low areas but lost in Gli1/low/p-MEK/high areas, suggesting that primary cilia have a positive correlation with Hedgehog signaling and an inverse relationship with Ras/MAPK. Furthermore, inhibition of primary cilia formation with ciliobrevin D or with a small interfering RNA to OFD1 (essential for cilia formation) both resulted in the inhibition of Hedgehog pathway activation.

- **c. Ras/MAPK activation occurs in treatment-resistant basal cell carcinomas that express high levels of Hedgehog pathway genes:** when comparing RNA sequencing data using gene set enrichment analysis from treatment-resistant to sporadic naive BCCs from the same patient, the authors did not identify Ras pathway activation in resistant BCCs. However, when distinguishing BCCs based on Hedgehog signaling levels, they found an inverse relationship between Hedgehog signaling and Ras singaling—Ras signaling was significantly enriched in cells with low levels of Hedgehog signaling. The authors propose that Ras activation occurs as an alternative rather than an additional mechanism to Hedgehog activation in resistant BCCs.

- **e. Ras/MAPK pathway activation confers resistance to MEK inhibitors in Smo inhibitor-resistant basal cell carcinomas:** the authors found that Ras/MAPK pathway activation in a Smo inhibitor-sensitive cell line (3T3) or murine and human basal cell carcinoma cell lines (ASZ and UW BCC cell lines, respectively) confers resistance to canonical and noncanonical Hedgehog pathway inhibitors. However, resistance to Smo inhibitors could be overcome by inhibiting the Ras/MAPK pathway with the MEK inhibitor UO126, which could be a promising therapy to prevent resistance.

**REFERENCES**


