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Hedgehog Signaling Inhibitors Fail to Reduce Merkel Cell Carcinoma Viability

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TO THE EDITOR

Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine skin cancer. Surgical resection and radiotherapy can successfully treat localized disease, but treatments for advanced MCC are needed.

Aberrant activation of the Hedgehog (Hh) signaling pathway plays a role in several cancers, including basal cell carcinoma (BCC) and medulloblastoma (MDB) (Gupta et al., 2010). One study has reported increased immunohistochemical staining of Hh pathway components (Hh ligands, PTCH, SMO, and Gli family members) in MCC tumors, suggesting that Hh pathway activation plays a role in MCC pathogenesis (Brunner et al., 2010). This resulted in speculation that Hh inhibitors may be effective in treating MCC (Li et al., 2011).

However, this hypothesis conflicts with mouse studies in which genetic activation of Hh signaling in the Merkel cell lineage failed to produce neuroendocrine skin tumors (Peterson et al., 2015; Xiao et al., 2015). Additionally, the loss-of-function *PTCH1* mutations associated with Hh-driven cancers are largely absent in MCC (Cimino et al., 2014; Harms et al., 2015); the rare *PTCH1* variants that have been reported in MCC (Goh et al., 2016) have unknown functional significance.

To characterize the extent of Hh pathway activation in MCC, we analyzed mRNA microarray expression data from MCC tumor samples compared with BCC and Sonic Hedgehog (Shh)-subgroup MDB samples (see Supplementary Materials and Methods online). All 23 MCC samples showed low expression levels of target genes indicative of Hh pathway activity, including *GLI1* and *PTCH1*, and obligate mediators of Hh signaling, such as *SMO* and *GLI2*. In comparison, Hh-driven BCC and MDB tumors showed marked up-regulation of these genes (Figure 1). This contrast, also noted in another comparison of MCC and BCC (Harms et al., 2013), suggests that active Hh signaling is not present in MCC.

To further investigate the potential role of Hh signaling in MCC, we treated the MCC cell lines Mkl-1 and WaGa with the SMO inhibitors cyclopamine and itraconazole and the Gli inhibitor GANT61. The Hh-driven BCC cell line UW-BCC1 (Noubissi et al., 2014) and the HeLa cell line, shown to be insensitive to Hh-pathway inhibition (Samarzija and Beard, 2012), were used as positive and negative controls, respectively. All three drugs showed a similar dose-dependent inhibition of BCC viability (Figure 2). In contrast to its effect on BCC cells, the classic SMO inhibitor cyclopamine failed to decrease MCC cell viability. The alternative SMO antagonist itraconazole showed a dose-independent reduction of cell viability in the Mkl-1 cell line, but it similarly inhibited HeLa cells, suggesting its effects were not mediated by antagonizing Hh signaling. Itraconazole did not significantly affect WaGa viability. The Gli inhibitor GANT61 reduced the viability of both WaGa and HeLa cells at higher concentrations, again suggesting off-target toxicity. In Mkl-1 cells, GANT61 failed to alter viability. Taken together, these data suggest that Hh pathway activation is not necessary for MCC viability and that antagonists of Hh signaling are unlikely to be effective treatments for MCC.

These findings argue against testing Hh signaling inhibitors in patients with MCC. However, we encountered a patient who was diagnosed with MCC while taking a SMO inhibitor. A 73-year-old man developed a mass in his left axilla 2 months after beginning vismodegib for multiple locally advanced BCC tumors on his bilateral trunk. The patient did not have nevoid basal cell carcinoma syndrome, and his past medical history was most significant for seizure disorder, multiple skin cancers, and mesothelioma. His medications included once-daily 150 mg oral vismodegib, phenytoin, propranolol, triamterene/hydrochlorothiazide, atorvastatin, gabapentin, docusate, and a multivitamin.

While taking vismodegib, the patient experienced mild muscle cramps and had reductions in the sizes of his BCC lesions. However, a semiannual

Abbreviations: BCC, basal cell carcinoma; Hh, Hedgehog; MCC, Merkel cell carcinoma; MDB, medulloblastoma; SCC, squamous cell carcinoma; Shh, Sonic Hedgehog

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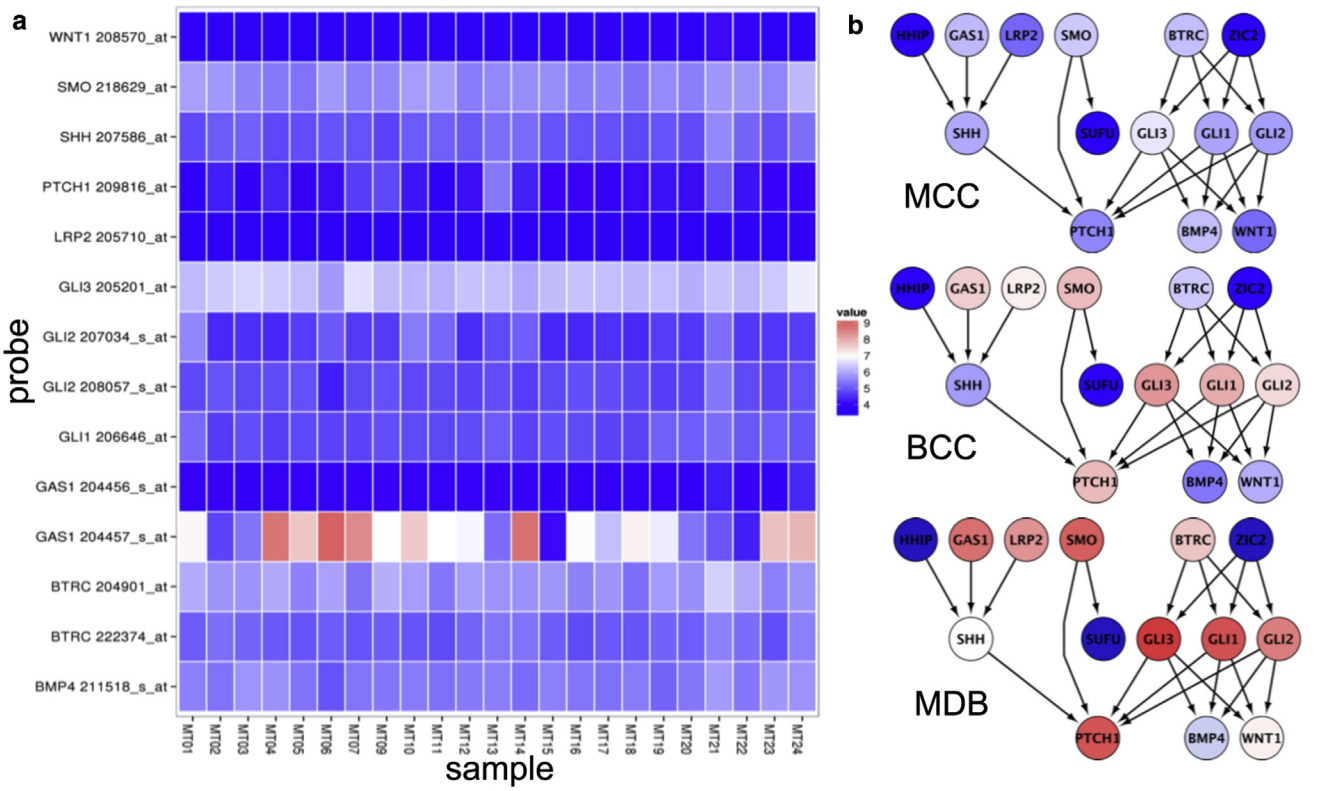

Figure 1. MCC lacks the mRNA expression signature of Hh pathway activation. Analysis of microarray expression data of Hh pathway genes in MCC tumors as well as in the Hh-driven cancers BCC and Shh-subgroup medulloblastoma. Red denotes high levels of mRNA expression, and blue denotes low levels of expression. (**a**) Expression heatmap generated for Hh signaling genes in 23 MCC tumors. All samples show low expression levels for most probes examined, including canonical Hh target genes *GLI1* and *PTCH1*. (**b**) Hh pathway schematic showing a comparison of Hh signaling gene expression between MCC and Hh-driven cancers. MCC lacks the up-regulation of Hh target genes and mediators of Hh signaling observed in BCC and Shh-subgroup MDB. BCC, basal cell carcinoma; Hh, Hedgehog; MCC, Merkel cell carcinoma; MDB, medulloblastoma; Shh, Sonic Hedgehog.

mesothelioma surveillance computed tomography scan identified the new axillary mass. Fine-needle aspiration showed malignant neuroendocrine cells that stained positive for cytokeratins (AE1/AE3), CK7, CK20, synaptophysin, and chromogranin and were negative for TTF-1 and LCA. The patient was diagnosed with stage IIIb MCC with unknown primary. His vismodegib treatment was discontinued.

A computed tomography scan performed 2 months after cessation of vismodegib showed a notable decrease in the size of the patient's axillary tumor, from 5.8 × 3.1 cm to 3.0 × 2.3 cm. A subsequent lymph node dissection showed a high-grade neuroendocrine neoplasm that stained positive for synaptophysin and CK20, consistent with his diagnosis of MCC. A course of adjuvant external beam radiotherapy was discontinued after 4 weeks because of local complications. The patient remained free of MCC for 3.5 years until he died from progression of his mesothelioma.

The course of this patient's MCC shows that MCC can arise in the setting of SMO inhibition, further supporting the conclusion that Hh activation is not a driver of MCC. The fact that the patient's MCC regressed after discontinuing vismodegib suggests the possibility that Hh signaling antagonists may even promote MCC growth. Vismodegib has been reported to increase the risk of squamous cell carcinomas (SCCs) (Mohan et al., 2016), and SCCs have also arisen within responding BCC tumors during vismodegib treatment (Ransohoff et al., 2015; Zhu et al., 2014). This has been attributed to activation of the Ras–mitogen-activated protein kinase pathway within BCC cells where the Hh pathway is inhibited, generating a resistant squamous cell tumor (Zhao et al., 2015). An analogous activation of an MCC driver pathway in an Hh-inhibited BCC tumor could similarly be responsible for this patient's MCC tumor.

Although vismodegib therapy was associated with tumor growth in this case, the MCC cell lines in our in vitro study did not experience enhanced growth in response to Hh signaling inhibitors. Spontaneous regression after biopsy is occasionally seen in MCC and provides an alternative explanation for tumor regression unrelated to Hh inhibition. Future studies will be needed to further examine the relationship between vismodegib therapy and MCC development. However, based on the lack of Hh target gene up-regulation, the absence of meaningful responses of MCC cell lines to Hh pathway antagonists, and the occurrence of an MCC tumor in a patient being treated with an Hh signaling inhibitor, we suggest that Hh inhibition would not be an effective therapy for MCC.

All human research was conducted in accordance with approved