Sonic hedgehog signaling pathway and gallbladder cancer: targeting with precision medicine approach

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Gallbladder cancer is a rare disease with only about 20% of cases diagnosed in early stages. According to the American College of Surgeons/American Cancer Society National Cancer Database in the AJCC Cancer Staging Manual [2010], 5-year survival rate is only 28% for patients with stage II gallbladder cancer and single digit in stage III/IV gallbladder cancers (http://www.cancer.org/cancer/gallbladdercancer/detailedguide/gallbladder-survival-rates). It is managed by radiation therapy and chemotherapy for patients with unresectable disease in late stages and no targeted therapy are available. Thus, novel treatment approaches are needed in gallbladder cancer.

The importance of sonic hedgehog (sHh) signaling has been widely recognized among oncologists since the 2012 approval by the US Food and Drug Administration (FDA) of vismodegib (GDC0449; Roche), a small molecule anti-smoothened (SMO), for the treatment of advanced basal cell carcinoma (BCC) (1). Besides, vismodegib has been shown activity in a subset of medulloblastoma (2). In 2015, another SMO antagonist sonidegib (LDE225; Novartis) was approved for use in the treatment of locally advanced BCC that has recurred following surgery or radiation therapy, or those who are not candidates for surgery or radiation therapy. The success was largely based on the genetic alterations of *Patched (PTCH) 1* gene, a tumor suppressor, in sporadic BCCs. Notably, sporadic BCCs were shown to carry a greater mutational burden including *PTCH1* mutations in 75% of tumors, which are nonsynonymous alterations with the predominance of nonsense and splice site mutations, as well as frameshift deletions and missense mutations. These mutations caused a complete/partial loss of function of one copy of the *PTCH1* gene (3).

Consequently, the suppression of PTCH1 on SMO is lost, leading to the constitutive activation of the Hh signaling via unleashing and accumulation of SMO and activation of its downstream transcription factor Gli1.

In addition to the mutation-driven ligand-independent Hh pathway activation, the ligand-dependent signaling by both autocrine and paracrine mechanisms are important to the sHh signaling-mediated tumor growth (4,5). The autocrine signaling refers to the mode that Hh ligand produced by tumor cells stimulates the Hh signaling in tumor cells; and the paracrine signaling is regarded as the one that tumor cell produced-Hh ligand activates stromal and endothelial cells, which produce growth factors in microenvironment to support tumor growth and survival. Mainly based on the paracrine mechanism of action, clinical trials were conducted in a randomized fashion— standard of care (SOC) as a control arm versus experimental treatment with SOC plus vismodegib in advanced colorectal carcinoma (6,7). The US National Cancer Institute (NCI) sponsored two other randomized trials based on the autocrine mechanism of action in pancreatic and gastric cancer (8,9). However, none of these trials met the clinical endpoints. There may be multiple reasons for the negative results, with the most reasonable explanation of the lack of predictive biomarkers. Wadhwa *et al.* have looked at the predictive biomarkers to trimodality therapy in esophageal cancer. Pretreatment nuclear Gli1 labeling index (Gli-1 Lis) was significantly associated with pathological stage progression (10). They hypothesized that Gli-1 Lis can be explored as a predictive biomarker for targeting the Hh pathway and other treatment approaches.

In gallbladder cancer, Hh pathway activation was

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confirmed by Matsushita *et al.* examining 37 gallbladder cancer specimens, in which SMO, sHh, or Gli1 expression was detected in the cytoplasm/nucleus of the cancer cells by immunohistochemistry (11). Their results were consistent with the findings by Li and colleagues (12), which first reported the Hh signaling activation in gallbladder cancer. Matsushita *et al.* further studied the effect of pharmacological inhibition of Hh pathway using cyclopamine and small interfering RNA (siRNA) on the Hh signaling to inhibit tumor invasion and epithelia-mesenchymal transition (EMT) (13). Unfortunately, the results using this pharmacological approach were not compelling possibly due to cyclopamine being less potent than vismodegib or sonidegib and having off-target effects. Additionally, Xie *et al.* have reported that the Hh pathway activation was important in the initiation of gallbladder cancer (14), and strong Gli1 expression was associated with poor survival in patients.

The clinical role of Hh pathway inhibitors in biliary tract cancers has not been evaluated. The experience from gastrointestinal malignancies, including pancreatic, colorectal and gastric cancers would argue that without using a predictive biomarker for patient selection, targeting Hh pathway is less likely to be successful in gallbladder cancer. Nevertheless, it warrants preclinical testing using SMO inhibitors other than cyclopamine in gallbladder cancer models *in vitro* and *in vivo*. It would be interesting to conduct a proof of principle clinical trial using Gli-1 as a biomarker for patient selection. Li *et al.* recently reported the genomic landscape of gallbladder cancer, and revealed that the ErbB family signaling is altered frequently (36.8%) and no PTCH alterations were detected from 57 tumor samples (15). The data may suggest that genetic profile of gallbladder cancer is different from gastric cancer which harbors 16% of PTCH1 and 12% SMO genetic alterations (16). Therefore, consideration should be given from multiple angles that tackle more than two activating pathways in gallbladder cancer.

In summary, the findings by Matsushita and colleagues demonstrated the role of Hh signaling pathway on the invasion and proliferation of gallbladder cancer cells in addition to expression of Gli1 and other key molecules of the pathway in human gallbladder cancer specimens. The preclinical findings seem to justify the Hh pathway as a potential therapeutic target in gallbladder cancer. However, in our view, it may increase the odds of success for clinical targeting the Hh signaling pathway in patient population with Gli1-expressing tumors as a precision medicine approach. It warrants further development and establishment of Gli-1 immunohistochemistry suitable to clinical use.

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Footnote

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