Editorial

Activated status of Hedgehog pathway in oral squamous cell carcinoma (OSCC): the door is still open

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The Hedgehog (Hh) signaling pathway is essential for regulation of cell differentiation and organ formation in a concentration-dependent manner during embryonic development. Also, this pathway is important to the maintenance of adult stem cells. Studies have shown that three members of this family are present in mammals: Sonic hedgehog (SHH), Desert hedgehog (DHH) and Indian hedgehog (IHH), all of which encode secreted proteins (1).

In the absence of a ligand, the Hh signaling pathway is inactive in those Hh responsive cells. In this case, the transmembrane protein receptor Patched (PTCH1) inhibits the activity of Smoothened (SMO), a transmembrane protein. The transcription factor GLI1, a downstream component of Hh signaling, is prevented from entering the nucleus through interactions with cytoplasmic proteins, including Fused (FU) and Suppressor of fused (SUFU), resulting in a repression of the transcriptional activation of Hh target genes. Activation of the pathway is initiated through binding of any of the three mammalian ligands (SHH, DHH or IHH) to PTCH. Ligand binding results in de-repression of Smo, thereby activating a cascade that leads to the translocation of the active form of the transcription factor GLI into the nucleus. Nuclear GLI activates target gene expression, including *PTCH1* and *GLI1* itself, as well as *HHIP*, an Hh binding protein that attenuates ligand diffusion (2). Other target genes important for the oncogenic function of the Hh pathway are genes involved in controlling cell proliferation [*CCND1*, CCNE1, *MYC* and components of the epidermal growth factor (EGF) pathway], in angiogenesis [components of the platelet-derived growth factor (PDGF) and vascular epithelial growth factor (VEGF) pathways] and in cell migration and invasion (activation of SHH signaling pathway is directly involved in lymphangiogenesis, activation of EMT through MMP-9) (3). This regulation of migration and invasion has been found in pancreatic cancer, lung cancer, gastric cancer and hepatocarcinoma (4). This feature has been strengthened with *in vitro* and *in vivo* experiments using GLI inhibitors and/or SHH transfection in various cell lines to study adhesion, migration, and invasion of tumor cells.

Oral squamous cell carcinoma (OSCC) is the most prevalent epithelial tumor of the head and neck region, and is characterized by a high occurrence of locally invasive growth and cervical lymph node metastasis, important factors for oral cancer patient prognosis. Fan *et al*. studied the SHH and GLI1 expression in OSCC by immunohistochemistry (IHC) and correlated this expression with clinical parameters, with EMT markers such as MMP-9 and E-cadherin, and prognosis (4). This article constitutes the first evidence that describes the role of Hh signaling pathway in migration and invasion for OSCC.

Both SHH and GLI1 were found with a significantly higher expression in OSCC than in non-cancerous oral mucosa. There was a significant positive correlation between SHH and GLI1 overexpression in OSCC with lymph node metastasis, among other associations. MMP-9 and E-cadherin are EMT-related marker proteins. The MMP-9 overexpression and E-cadherin downregulation

have previously been associated with an increase in tumor invasion and metastasis. A similar expression pattern of E-cadherin and MMP-9 expression was consistently found in OSCC tissues, with a high correlation with lymph node metastasis (P<0.05 for both), among other clinical features. Survival analyses also made sense because patients with high GLI1 and MMP-9 protein expression had lower 5-year survival rates than those with low levels of these proteins (P<0.05). Those with low levels of E-cadherin protein expression had a lower 5-year survival rate than those with high levels (P<0.05). Spearman's correlation test revealed that E-cadherin expression had a significant negative correlation with SHH and GLI1 protein expression, and that, conversely, GLI1 expression was positively correlated with MMP-9 protein expression, suggesting that abnormal activation of Hh pathway (via SHH and GLI1) has a putative role in MMP-9 and E-cadherin in order to induce EMT in OSCC. This effect can be caused by GLI1, which can increase expression of the transcription factor SIPI, promoting expression of two EMT-related transcription factors, TWIST2 and SNAI2 (5).

Recent reports have stated that SHH signaling mediates invasion and metastasis through its interaction with the ERK and PI3K/AKT pathways. Hyperactivity of the EGFR/(MAPK) ERK and PI3K/AKT/mTOR pathways are reported abnormalities of advanced oral and oropharyngeal SCC (6), but the relationship of these pathways specifically with the angiogenesis process is not fully understood, suggesting that this is a very interesting approach to be studied in OSCC in order to clarify the activation status of all proteins related to ERK and PI3K/AKT pathways and to assess whether the downstream effector of these pathways such as mTOR, P70S6K1, 4E-BP1 or hypoxia-inducible factor (HIF) are then triggering these angiogenesis processes. Data from the Fan group's indicate that MMP-9 expression may be induced by the PI3K/AKT pathway to cause angiogenesis in OSCC; thus, subsequently, this SHH signaling activation pathway may also contribute to angiogenesis in OSCC.

The increase in GLI1 expression was accompanied in many cases by an elevation of SHH; however, whether this enhanced expression of GLI1 was entirely caused by abnormal activation of SHH signaling is unclear because some studies have suggested that GLI1 protein changes were not immediate outcomes of SHH signal transduction, but rather were subsequent events mediated by GLI1-driven transcription (4,7). Therefore, in order to elucidate this issue, other molecules or upstream modulators such as PTCH1, SMO, DHH or IHH could be the focus of future analyses.

As GLI1 seems to be a key effector in Hh signaling, this molecule or a member of its family, constitute promising prognostic markers and potential therapeutic targets in OSCC. In 2011, Yan *et al.* offered an interesting model for studying GLI signaling inhibitors. They found that GLI2 expression, another transcriptional activator of Hh/GLI signaling, was present in 44% of samples (60) and was significantly associated with poor clinical outcomes. Only 44% of the patients whose tumors expressed GLI2 survived at 5 years after surgery compared to 77% of those whose tumors lacked the GLI2 expression (P<0.0001). They also established a model based on two Hh/Gli inhibitors, cyclopamine and GANT61, which could effectively inhibit GLI expression, decrease cell growth, promote G1 arrest, increase apoptosis, and inhibit migration of OSCC cell lines, demonstrating not only that activation of this pathway is important in OSCC progression, but also that a subset of OSCC patients may benefit from anti-Hh/GLI therapies (7). These drugs could be studied to determine their effects on the expression of proteins in the Hh pathway or another pathway, or to determine their results in other cell features in either *in vitro* or *in vivo* models.

In addition, recent findings suggest that Hh signaling may also promote tumorigenesis in a paracrine manner from the tumor to the surrounding stroma, or in cancer stem cells (CSCs) (8). As oral mucosa is continuously exposed to environmental forces and needs to be constantly renewed, its epithelium contains a large reservoir of epithelial stem cells, which can withstand strong stress mechanisms. Better purification of the stem-like cell population in oral carcinomas is necessary to clarify which metastatic characteristics are indeed unique to these cells. Taking advantage of this, some research groups have designed *in vitro* and *in vivo* models of metastasis to study the behavior of this unique tumor cell subpopulation in head and neck squamous carcinoma (HNSCC). As CSCs possess a greater capacity for tumor growth and metastasis compared to non-CSCs, it is thought that CSCs may be those mainly responsible for the development of metastasis in HNSCC. There is growing evidence that CSCs behavior is orchestrated *in vivo* in tissue-specific, "niche" microenvironment that supports stem cell maintenance and resistance to anoikis, suggesting that targeting the crosstalk between CSCs and other cells from their supportive niche may provide an effective way to abrogate the tumorigenic function of these cells and to trigger EMT. However, it remains unclear how CSCs carry out the metastatic process in these carcinomas and how the metastatic behavior of OSCC is modulated by CSC phenotypic characteristics (9,10). Here is where paracrine

secretion of Hh pathway modulators might be important in the understanding of these tumorigenic or spreading mechanisms. Whether Hh pathway regulators have such an implication in OSCC, the inhibitors against these modulators could be tested on these OSCC models in order to create novel therapeutic approaches that will result in significant improvement for the management and outcome of patients with this disease.

In conclusion, the door remains open for further advances in the knowledge of the role of the Hh signaling pathway in OSCC, considering at least four points. First, the implication of other pathways or other members of the Hh pathway (i.e., DHH, IHH, among others) in the activation of either GLI1 or other transcription factors in OSCC. Second, acquiring a better understanding of the relationship between the Hh and angiogenesis pathways (mTORC1/HIF/VEGF). Third, the use of new chemical inhibitors against the Hh pathway on *in vivo* and *in vitro* models in order to accelerate the potential treatment with these drugs in OSCC patients. Fourth, elucidating the potential relationship between the paracrine secretion of Hh ligands into the niche microenvironment of CSCs and the subsequent result of an invasive OSCC. Any progress in these areas could be useful for enhancing the current treatment protocols in OSCC and likely improving patient prognosis.

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Footnote

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