

## Hedgehog signaling pathway: the must, the maybe and the unknown

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### ABSTRACT

Lung cancer treatment consists from the basic chemotherapeutic drugs (e.g., platinum analogues) and from pharmaceuticals targeting the different genome of lung tumors (e.g., tyrosine kinase inhibitors). During the last years pharmaceuticals targeting the tumor mutations are approved for first line treatment since they have provided increased overall survival in comparison to standard chemotherapy treatment. Furthermore, due to the increased interest in unrevealing the mechanisms of cell mutation, tumor evolution and tumor cell maintenance the hedgehog pathway has been elicited. Along with Notch and Wnt these three pathways are responsible for progenitor cell development and pulmonary organogenesis. Inhibitors of this pathway have been discovered and their application in the clinical practice is being investigated. However, further understanding of the mechanisms of regulation is needed.

### KEY WORDS

Hedgehog; molecular pathways; lung cancer

*J Thorac Dis* 2013;5(2):195-197. doi: 10.3978/j.issn.2072-1439.2013.03.02

The last decade should be addressed as the era of the genome. Several therapies targeting specific mutations to lung cancer have been approved as first line treatment (1,2). However, tumor is the product at the last stage of a process that started several steps before. Therefore an increasing effort is being made to identify all the implicated pathways and mechanisms. This effort has elicited the hedgehog (Hh) pathway which was first observed in *Drosophila* larva (3). The Hh is responsible for regulating the embryonic stem cells in the early life development of the respiratory system. Further factors included in the normal lung branching of the respiratory system are: (I) bone morphogenic protein (BMP), (II) fibroblast growth factor (FGF) and Ras modifiers. These three additional mechanisms have been well identified in a *Drosophila* model (4). The first observation between cancer and Hh was observed with studies of Gorlin's Syndrome. The activation of Hh is observed in many cancer

types; skin, brain, gastrointestinal, breast, prostate (5) and recently small cell lung cancer (SCLC) (6,7) and non-small cell lung cancer NSCLC) (8-11). In the study by Raz *et al.* the Hh signaling pathway was evaluated along with the aldehyde dehydrogenase family 1 member A1 (ALDH1A1) in stage I-II NSCLC (10).

The Hh pathway is a mechanism of repair/regeneration in adult respiratory tissue, and is deregulated with smoking (12). Since SCLC is observed in heavy smokers it is evident why Hh pathway deregulation induces mostly in this type of cancer tumorigenesis. The Hh signaling is responsible for the formation of the respiratory system (13-15), it has been observed that when cyclopamine and jervine where administered in pregnant animals the respiratory system formation presented abnormalities (16). Hh is responsible for the main structure of the respiratory system, regarding the respiratory epithelium which consists of basal cells, beating cilia and clara cells their regulation is performed through the Notch and Wnt signaling (17). These three mechanisms continue to work as a cluster to repair and regenerate the injured respiratory system, only in the epithelial progenitor cells. The persistent airway epithelial injury activates the Hg signaling and promotes the expansion of the airway epithelial progenitor cells (17). It has been previously observed in surgically resected patients with NSCLC that the Hh signaling pathway was activated and overexpressed in the tumor while in the normal lung tissue such observation was not made (8). The Hh signaling

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Submitted Feb 07, 2013. Accepted for publication Mar 07, 2013.

Available at [www.jthoracdis.com](http://www.jthoracdis.com)

ISSN: 2072-1439

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consists of three variants; desert (Dhh), Indian (Ihh) and sonic (Shh). The Shh is formed after a 45 kDa protein is converted to a 19 kDa (18). In addition, there are two receptors; the transmembrane receptor-patched homolog 1 and 2 (Ptch 1 and 2), and a G protein coupled receptor the smoothed (Smo). Finally it consists of a cytoplasmic complex which the glioma-associated oncogene homolog (Gli) family (19). The Shh and Gli 1 are expressed in lung cancer both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). However, it has been observed that both Shh and Gli are observed in 50% of SCLC and only in 10% of NSCLC (17). Gli 3 has been found to be expressed in NSCLC cancer cell lines (7), and in one study Gli 2 was found also expressed (8). Gli target molecules have been extensively analyzed for their connection with squamous cell carcinoma. Out of 223 genes the 37 were associated with squamous cell carcinoma. CDC45, CDK1, CSTA, HDAC1, JAG1, JUP, KRT15, KRT17 have been found up-regulated and CXCL2, FHL1, FOXA2, PAPSS2, RHOB, SAP18 and SYNE1 were down-regulated (9). All the dysfunctional genes are responsible for the deregulated production of enzymes, growth factor, cytokines, kinases, transcription regulators and transporters. These findings were found to differ among different cell lines (7) and the inhibition of Hh was observed only in SCLC and not NSCLC, because in SCLC both Shh and Gli are expressed (17). It has been observed that genetic instability of cancer cell lines may cause phenotypic changes (7). A possible explanation is that in NSCLC the Hh is regulated by other independent mechanisms (8). Hh activation was also observed higher in squamous cell carcinoma (8,9). The Hh interacting protein (HIP) inhibits the Hh pathway however it is down-regulated by neo-angiogenesis which occurs in malignant transformation and evolution. Malignant cell proliferation is maintained once the mechanism of Hh regulation has been diverted. Ectopic expression of Shh leads to ectopic Hh interacting protein (20). In the study by Olsen *et al.* (21) it was demonstrated that angiogenesis was associated with Hh deregulated by inhibition of HIP. Moreover, the Hh pathway is influenced by other relevant cancer pathways such as the PRKCD which activates the ERK signaling. In specific the ERK signaling up-regulates the Gli transcription (22). Recent studies have also identified that apart from the Wnt and Notch, the down-regulation of Hox genes A3 and B4 are Hh-dependent Stat 3 (23). Epidermal growth factor receptor mutation has been also associated with Hh overexpression (24). Forkhead Box a family of transcription factors responsible for cell proliferation (25) was investigated for association in the Hh pathway signaling (8). It was observed that FOXM1 which is involved in the carcinogenesis of many human tumors is associated with Gli 1, Gli 2, SMO, PTCH 1 and Shh in NSCLC (8,26). FOXM1 maybe one of the factors affecting the Hh activity specifically in NSCLC. However, correlations in tissue

have to be made along with other mechanisms stated in the current published literature.

Furthermore, interaction of the 7<sup>th</sup> membrane spanning receptor (or smoothed SMO) was observed in association with Ras related protein (Rab)-23, platelet derived growth factor receptor- $\alpha$  (PDGFR- $\alpha$ ), hepatocyte nuclear factor 3-beta (HNF3 $\beta$ ) (27). The SMO is localized in the cytoplasm and cancer cell membranes, however it is not able on its own to activate abnormal signal activation. Rab-23 is involved in the function of Gli in the nuclei, but not in the cytoplasm (27). Gli 1 which is found overexpressed in SCLC activates PDGFR- $\alpha$ , this observation demonstrates that this receptor is under the control of Hh signaling (27). The HNF3 $\beta$  may act as an independent factor for elevating Shh expression (27).

Stem cells have the ability to continuously regenerate and provide differentiated progenies. However, it is well known that a deregulation of this process with the additional influence of activated signaling pathways may induce malignant transformation (6). Microenvironment control is therefore essential. The current published literature indicates that the Hh signaling pathway is activated by continuous injury of the respiratory epithelium which is more common in heavy smokers. Therefore several groups have demonstrated an association between Hh deregulation of the Hh cascade in SCLC and squamous cell carcinoma which are both strongly associated with smoking. Further investigation towards the molecules produced from the Hh deregulation such; cytokines, enzymes and growth factors and their interaction with the Hh pathway are warranted. Finally, monoclonal antibodies are currently being developed. Cyclopamine and jervine are promising inhibitors of Hh pathway (17,28). Other molecules under evaluation are KAAD-cyclop, SANT1-4, CU61414, GANT 61, Tyrosine kinase inhibitors, p70S6K2casein kinases (CK1a and CK1e) glycogen synthetase kinase 3 $\beta$  (GSK3 $\beta$ ) and protein kinase A (PKA) (e.g., foskolin), however resistance to this molecules has been observed due to differentiations in the Hh pathway due to mutation and cancer evolution (9,11,24,29-35). Targeting the cancer stem cells we may have the advantage of avoiding chemotherapy toxicity and impede a fundamental way to cancer evolution and maintenance.

## Acknowledgements

*Disclosure:* The authors declare no conflict of interest.

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**Cite this article as:** Zarogoulidis P, Zarampouka K, Huang H, Darwiche K, Huang Y, Sakkas A, Zarogoulidis K. Hedgehog signaling pathway: the must, the maybe and the unknown. *J Thorac Dis* 2013;5(2):195-197. doi: 10.3978/j.issn.2072-1439.2013.03.02