Editorial

New insights into the functional consequences of ephrin A3 mutations in non-small cell lung cancer

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Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer-related deaths worldwide (~1.4 million deaths in 2008) (1). Non-small cell lung cancer (NSCLC) is the most common lung cancer histological subtype (~85% of all lung cancers) and 50% of these tumors are adenocarcinomas. The identification of the driver events in NSCLC is under intense investigation, aided by the emergence of next-generation sequencing technologies. The incidence and roles of oncogenic mutant EGFR and kRas proteins have been well described (2). More recently ALK, RET and ROS1 fusion proteins have also been identified utilizing these next-generation methods, and these molecules are under concerted investigation as potential therapeutic targets to improve clinical outcomes (3-5). However, there are a growing number of genes, mutated in up to 10% of lung cancers, for which their function in lung cancer is poorly understood. Thus, there is a significant need to elucidate the effects of these cancer-associated mutations in lung cancer. Recently, Zhuang and colleagues highlighted the role of ephrin A3 receptor (EphA3) mutations in lung cancer (6).

The Eph receptors make up the largest subfamily of receptor tyrosine kinases, with 16 known receptors (14 in mammalian systems) (7). These receptors are activated by membrane-associated ligands termed ephrins. Initial insights into the functions of the Eph signaling axis were largely derived from studies in the central nervous system, where many of the Eph kinases are abundantly expressed and affect vascular development, tissue-border formation, cell migration, axon guidance and synaptic plasticity (8). Recent studies have demonstrated alterations in the expression of both Eph receptors and ephrin ligands in numerous cancer types. The ephrin/Eph receptor signaling axis has been associated with tumor growth, invasiveness, angiogenesis and metastasis both *in vitro* and *in vivo* (9). Previous studies from our laboratory (10,11) and other investigators (8) have demonstrated that the activation of certain ephrins and Eph receptors promotes glioma cell migration and invasion.

The overexpression or deregulation of receptor tyrosine kinases, such as EGFR, is generally associated with cell transformation. Interestingly, alterations in Eph receptor expression has been linked to both pro- and anti-tumor effects across different cancer cell contexts. While the function of EphA3 in specific cancer contexts is still under investigation, there is growing evidence that EphA3 may act as a tumor suppressor in some cancer types, modulating tumor invasiveness (6,12,13). Somatic mutations in EphA3 have been identified in hepatocellular carcinoma (14), melanoma (15), pancreatic carcinoma (15,16), glioblastoma (15), breast cancer (15,16), and more recently in lung cancer (16-19). Mutations have been identified over the length of the EphA3 receptor including the extracellular ligand binding and receptor-receptor interface domains and the intracellular kinase domain. However, the impact of these mutations on EphA3 receptor function and their role in lung cancer pathobiology has not been defined. The report from Zhuang *et al*. in the *Journal of the National Cancer Institute* is the first to investigate the functional consequences of specific EphA3 receptor mutations in lung cancer.

Zhuang and colleagues report several lines of evidence that support a role for EphA3 as a tumor suppressor in lung cancer (6). First, copy number analysis indicated that EphA3 was frequently deleted in a significant proportion of NSCLC cell lines. Second, Eph3A was also frequently deleted

in a large cohort of primary lung cancer tumor samples compared to matched control samples. Third, IHC analysis demonstrated that Eph3A expression was significantly reduced in tumor samples relative to nonmalignant tissues on a lung tissue microarray substantiating the results obtained with cell lines. Notably, the suppression of EphA3 expression was statistically significantly reduced across all stages of lung cancer progression compared to normal tissues. Thus, loss of EphA3 receptor expression suggested an inverse relationship to lung cancer. In addition to loss of receptor expression, the authors investigated the functional effect of multiple mutations in the EphA3 receptor that have been identified in lung cancer cells and tumor samples. Interestingly, many of these mutations were classified as loss of function mutations as they resulted in the reduction or loss of receptor kinase activity. Somatic mutations resulting in impaired kinase activity, reduced ligand binding and altered cell surface localization have been described in several tumor types (12). A gene expression signature associated with EphA3 mutation in lung cancer was highly prognostic of reduced patient survival.

To further support the role of EphA3 as a tumor suppressor in lung cancer, wild-type (WT) EphA3 was ectopically expressed in lung cancer cell lines with low endogenous EphA3 expression. Increased expression of WT EphA3 did not alter cell proliferation but suppressed cell survival *in vitro* and tumor growth *in vivo* in a murine xenograft model through inhibition of AKT activity and induction of apoptotic signaling (6). In contrast, ectopic expression of cancer-associated EphA3 somatic mutants did not inhibit AKT activity, which correlated positively with a loss of increased apoptosis and loss of inhibition of *in vivo* tumor growth relative to the WT EphA3. Interestingly, the co-expression of WT EphA3 and loss of function EphA3 mutants blocked the kinase activity of the WT receptor and abrogated the tumor suppressive activity of WT Eph3A *in vivo*, suggesting a dominant-negative action of the EphA3 mutants. Interaction of WT and mutant EphA3 was demonstrated through co-immunoprecipitation experiments, with the mutant EphA3 suppressing tyrosine phosphorylation of WT EphA3 in a dose-dependent manner. Thus, mutant EphA3 receptors encoded by lung cancer-associated somatic mutations could suppress the anti-tumor effects of WT EphA3 in lung cancer through the formation of non-functional heteromeric complexes. This mechanism provides an alternative to deletion of EphA3 to remove its anti-tumor suppression.

In conclusion, EphA3 is an often-mutated gene in lung cancer as well as several other tumor types. The work of Zhuang *et al.* describes EphA3 as a tumor suppressor in lung cancer, with receptor activity lost through gene mutation, gene deletion or reduction of receptor protein expression. The ability of mutant EphA3 receptor to act as a dominant-negative receptor in complex with WT EphA3 receptor offers the possibility that heterozygous mutation could have significant phenotypic impact in lung cancer. The mechanisms through which EphA3 is silenced may lead to novel therapeutic strategies in lung cancer. As the roles of distinct Eph receptors and ligands continue to display both pro- and anti-tumor effects in specific cancer cell contexts, a thorough understanding of specific Eph receptor signaling mechanisms and the development of specific Eph receptor therapeutics will be necessary for clinical utility. This work also clearly necessitates the need to functionally characterize recurrent mutations discovered in specific cancer types by next-generation sequencing.

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