Editorial Commentary

# *PI4K2A*: a novel potential therapeutic target for lung cancer

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Despite the advances in precision medicine, invasion, and metastasis continue to be the leading causes of cancer-related deaths. A better comprehension of the disease progression will decide new therapeutic strategies. Non-small cell lung cancer (NSCLC) is the deadliest cancer worldwide, primarily because of its high tendency to invade and metastasize (1). Therefore, preventing or treating metastasis is vital for ameliorating survival but requires a profound knowledge of the underlying drivers and pathways (2).

The tumor microenvironment (TME) is a critical component in cancer implicated in tumorigenesis and metastasis. Recently, it has been proposed that secreted peptides by malignant cells play a crucial role in triggering TME factors that favor invasion and metastasis (3). Among these factors, collagenase remodels collagen fibers, increasing stromal stiffness and facilitating cancer cell mobilization for invasion (4). However, clinical investigations targeting secreted peptides in the TME, such as matrix metalloproteinases (MMPs), did not demonstrate robust anti-cancer regression (5), contributing more as a physical and biochemical barrier, preventing drug assessment and immune response.

Moreover, acquired insights have linked genetic drivers and inflammation to the abnormal initiation of an epigenetic process known as the epithelial-mesenchymal transition (EMT), which confers metastatic ability to tumor cells (6) and resistance to therapy (7). EMT is a complex program modulated by multiple transcription factors, post-translational events, epigenetic modifications, and noncoding RNA-mediated regulation. Activation of EMT facilitates the replacement of polarized epithelial cells phenotype toward a mesenchymal phenotype, followed by the loss of epithelial characteristics, apicobasal polarity, intercellular junctions, and acquisition of inherent migratory and invasive traits (6). Together, genetic drivers of metastasis and epigenetic mechanisms related to EMT induce cancer invasion endowing elasticity and complacence to malignant cells, improving their survival throughout the metastatic journey (6).

Kundu and colleagues have demonstrated that the metastatic sequence and EMT are initiated by the loss of miR-200 and miR-96 in epithelial cells, allowing their *ZEB1* and *FOXF2* targets to activate mesenchymal cells to drive metastasis (8). However, inhibiting *ZEB1* or adding microRNAs (miRNAs) to control EMT for treating metastasis has proven to be a therapeutic challenge (8).

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This indicates that new experiments are necessary to dissect the EMT downstream pathway to inhibit metastasis and mesenchymal cells. In this regard, researchers have studied vesicle traffic of intracellular proteins to drive the malignant secretion of extracellular matrix-modifying factors leading to invasive tumor cell behavior (9-11).

Considering the recent study published by Tan *et al.* (11) brings out new information into the mechanisms that EMT coordinates exocytic and endocytic vesicular trafficking impacting NSCLC recurrence and metastatic progression. They identify the *PI4K2A* a membrane-associated RNA-dependent protein kinase, as a key player in cell signaling and vesicle trafficking, as a promise therapeutically actionable driver of lung cancer progression. *PI4K2A* kinase expression has long been associated with human diseases, including cancer, immunodeficiencies, viral infection, and neurodegenerative diseases (12,13). Nevertheless, its involvement in lung cancer has not been fully investigated.

Of note, previous studies showed that the submicroscopic Golgi organelle functions are modulated from *ZEB1*-mediated EMT to invasion of the tumors (9,10). Dysfunction of Golgi machinery and increasing vesicle trafficking are crucial characteristics of metastatic cells. These cells synthesize proteases, fibrillary collagen, and proteoglycans, into the extracellular space, preparing the TME for malignant invasion and metastasis (9,10). However, the mechanisms that regulate EMT-Golgi-metastasis and abnormal secretome remain poorly understood. Clinical treatment of lung cancer by inhibition of malignant Golgi secretion has proven ineffective (10). Improving our understanding of the drivers and pathways regulators of Golgi organelle functions and exocytosis during EMT would allow this machinery process to treat or prevent metastatic disease.

In line with these findings, *in vivo* and *in vitro* experiments showed that the EMT-activating transcription factor *ZEB1* triggers a PI4K2A-dependent mechanism resulting in a protein complex that promotes secretory vesicle synthesis within the Golgi apparatus, establishing a hypersecretory malignant scenario supported by prometastatic receptors such as osteopontin (*SPP1*) (11). The authors demonstrated that *PI4K2A* in the endosomal compartment creates an *SPP1*-dependent autocrine loop to inhibit lysosomal disintegration of the *AXL* gene, a driver of cancer cell migration (11). They concluded that EMT works as a central pivot in exocytic and endocytic vesicular trafficking, shaping a dynamic malignant hypersecretory environment that propels the progression of lung cancer (11).

Phosphatidylinositol 4-phosphate (PI4P) is widely recognized as a major regulator of vesicular trafficking, influencing intracellular signaling and metabolic pathways (12). Comprising types II and III, the phosphatidylinositol 4-kinases (PI4Ks) generate PI4P from phosphatidylinositol (PI) (13). The type III PI4Ks consist of two isoforms: PI4KIIIα (hereafter referred to as PI4KA) and PI4KIIIβ (referred to as PI4KB). While both PI4KA and PI4KB are peripheral membrane-binding proteins, the type II PI4Ks are membrane-binding lipids regulated by lipidation and post-translational modifications (14). The targeted recruitment and activation of PI4KA and PI4KB on membranes is crucial to their control. Elevated levels of PI4KB, PI4KA, and PI4K2A are correlated with unfavorable outcomes and malignant progression in various types of tumors (12,13). The chromosome 1q21.3 amplicon houses *PI4KB* and preserves malignant cell survival by activating a PI4KB-dependent secretome (14). Conversely, *PI4K2B* functions as a suppressor of malignant cell invasion and is often deleted or downregulated in human cancers (14). In contrast, *PI4K2A* fosters tumorigenesis by enhancing the stability of EGFR protein (15) and aiding in the transport of misfolded proteins to the lysosome, consequently supporting the survival of malignant cells (16). *PI4K2A* fortifies the stability of the AXL tyrosine kinase receptor, orchestrating the coordinated exocytic and endocytic movement of SPP1 and its receptors in mesenchymal lung cancer cells, triggering a pro-tumorigenic autocrine loop (11).

The investigation by Tan *et al.* (11) not only assigns innovative functions to *PI4K2A* but also raises intriguing questions that we addressed below.

Regarding metastasis prevention and cancer cure, while preventing metastasis is essential for improving survival, essentially curing lung cancer should be more critical. Does *PI4K2A* only play a role in metastasis, or is it involved in the primary development of lung cancer as well? *Figure 1* illustrates the *PI3K/AKT* pathway, involved in carcinogenesis. Following activation by receptor tyrosine kinases or *RAS*, PI3K phosphorylates PIP2, producing PIP3. This event activates *AKT* and *PDK*. *AKT* hinders *GSK3*, ensuring the stability of cyclin D1 and impeding p27, thereby promoting cell-cycle progression. *AKT* enhances cell survival by inhibiting the Bcl2-antagonist of cell death. Moreover, *AKT* regulates protein synthesis and cell growth by phosphorylating mammalian targets of rapamycin (mTOR), facilitating the translation of messenger RNA (mRNA) to synthesize proteins for cell growth (16). Note that *PI4K2A* not only plays a role in metastasis but is also

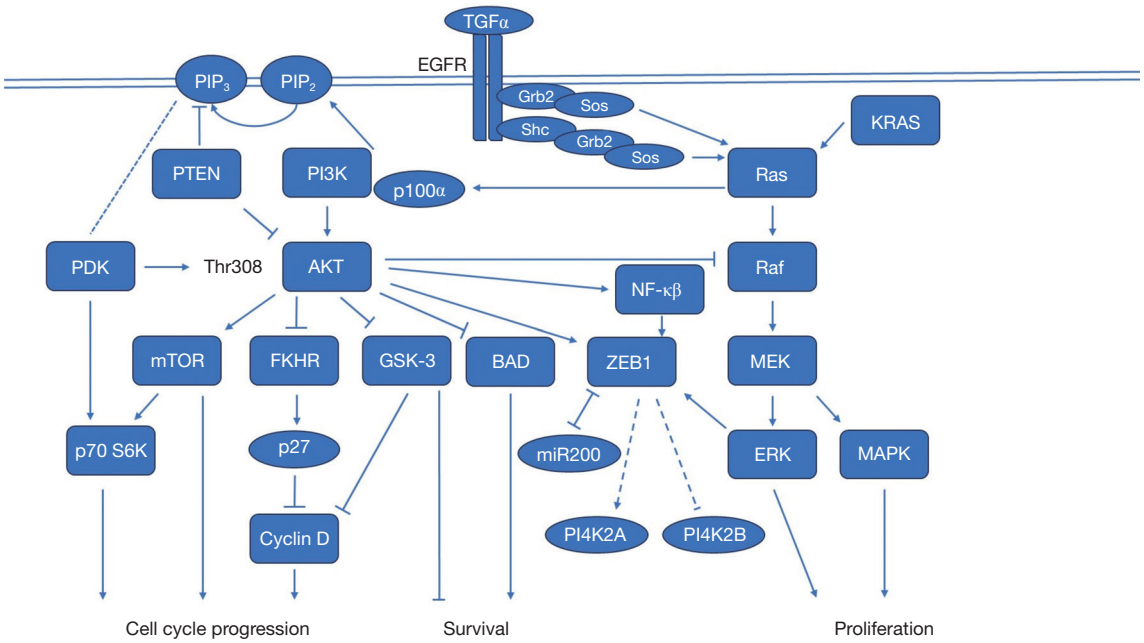


**Figure 1** PIK pathway and related pathways. Upon activation of receptor tyrosine kinases or *RAS*, they initiate signaling cascades that involve *PI3K*, leading to the phosphorylation of PIP2 and the generation of PIP3. These processes activate *AKT* and *PDK*. Once activated, *AKT* inhibits *GSK3* through stabilizing cyclin D1, a key player in cell cycle progression. *AKT* also impedes FKHR-mediated transcription of the Cdk inhibitor p27, promoting cell-cycle progression. Additionally, cell growth is influenced by the phosphorylation of p70S6K, regulated by the phosphorylation of mTOR. When activated by *AKT*, mTOR promotes the translation of mRNA, facilitating protein synthesis for cell growth. Another *AKT*-regulated process is the inhibition of the *BAD*, enhancing cell survival. *AKT* also activates *ZEB1*, which drives PI4P synthesis, subsequently impacting *PI4K2A* and *PI4K2B*. Molecules such as *RAS/ERK* and NF-κB trigger the expression of *ZEB1* proteins, targeting molecules like miR200. All these events and signaling pathways play crucial roles in cell cycle progression, survival, and proliferation. mTOR, mammalian targets of rapamycin; mRNA, messenger RNA; PIK, phosphatidylinositol kinase; Cdk, cyclin-dependent kinase; p70S6K, p70S6 kinase; PI4P, phosphatidylinositol 4-phosphate.

involved in the primary development of lung cancer as well (16). Therefore, inhibiting *PI4K2A* is crucial for metastasis and primary tumor control. As previously reported phosphatidylinositol 3-kinase inhibitor (LY294002) induces apoptosis of cancer cells *in vivo* and *in vitro* (17).

Is the expression of *PI4K2A* independent of known NSCLC driver gene mutations such as *KRAS* and *EGFR*? Simultaneous suppression of *EGFR* at both the protein and activity levels through the inhibition of PI4KIIα exerts an anti-tumor effect and promote the handling of misfolded proteins to the lysosome, thus preserving malignant cell survival (15). Some preclinical models emphasize the potential for targeting *PI4KA* and *PI4KB* in cancer (18-20). *PI4KA* initiates the PI4P pool formation at the plasma membrane, which undergoes phosphorylation to PIP2 and subsequently to the pro-development signal PIP3. In human cancer, frequently mutated genes such as *HRAS*, *NRAS*, and *KRAS* activate class IA PI3K p110α, generating essential PIP3 in *RAS*-driven tumorigenesis (21). The interaction between the PI4KA regulatory protein EFR3A and KRAS leads to decreased PI4P, PS, and KRAS levels at the cytoplasmic membrane, resulting in a concurrent reduction in oncogenic signaling and tumorigenesis when either PI4KA or EFR3A is interrupted (18). Treating mutant *KRAS* pancreatic cell cultures with a combination of a G12C-specific RAS inhibitor (sotorasib) and a *PI4KA* inhibitor demonstrated a synergistic inhibitory effect on cancer cell growth. In experimental pancreatic tumors, *PI4KA* and *EFR3A* were upregulated compared to normal tissue (19). Based on these findings, we deduced a beneficial yet narrow therapeutic window for *PI4KA* inhibition in mutant *KRAS*-driven cancers, especially when used in combination with either *PI3K* or *KRAS* inhibitors, as a meticulous analysis of toxicity becomes essential.

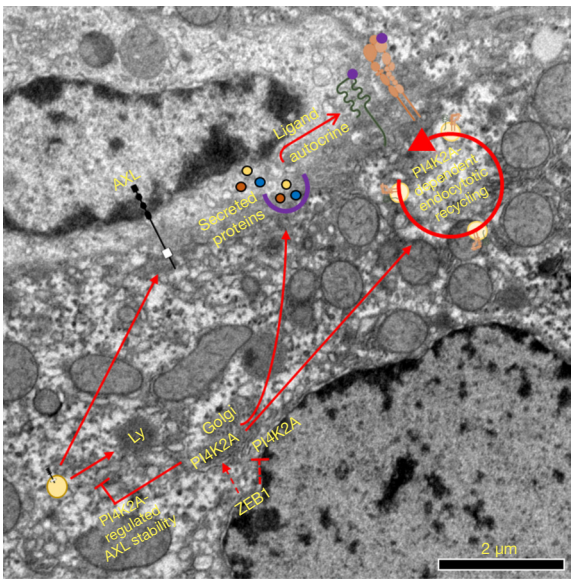


**Figure 2** *PI4KA* pathway simulated at TEM in a case of lung adenocarcinoma. Activation of *ZEB1* at EMT drive PI4P synthesis in the Golgi apparatus. Note the increase of Golgi cisternae near the nucleus. *PI4K2A* creates a receptor-dependent autocrine loop on the endosome to prevent lysosomal degradation of AXL receptor tyrosine kinase, driving cell migration. In this scenario, the vesicle transport processes involving both exocytosis and endocytosis establish a clinically exploitable hypersecretory condition that pushes the advancement of lung cancer. Magnification: 24,000×. Ly, lysosome; TEM, transmission electron microscopy; EMT, epithelial-mesenchymal transition; PI4P, phosphatidylinositol 4-phosphate.

The next question is related to miR-200-dependent EMT in molecular targeted therapy resistance. Preclinical investigations involving osimertinib-resistant lung cancer cells revealed an association between EMT, reduced miR-200 expression, and elevated *ZEB1* expression. Treating resistant clone cells with a histone deacetylase inhibitor before exposure helped mitigate resistance by reversing EMT (22). In another study using preclinical models with crizotinib-resistant lung cancer cells, EMT correlated with diminished miR-200c expression and increased *ZEB1* expression, leading to cross-resistance against new-generation *ALK* inhibitors such as alectinib, ceritinib, and lorlatinib. Pretreatment with the histone deacetylase inhibitor quisinostat effectively deactivated this resistance by reversing EMT both *in vitro* and *in vivo* (22). These findings suggest that inhibiting EMT enhances the efficacy of targeted therapy.

AXL has been reported as an initial resistance factor in molecular targeted therapy. A recent study revealed that while AXL-low expressing *EGFR* mutated lung cancer (EGFRmut-LC) cells exhibit greater sensitivity to osimertinib compared to AXL-high expressing EGFRmut-LC cells, a small population develops osimertinib tolerance. In AXL-low-expressing EGFRmut-LC cell-derived xenograft and patient-derived xenograft models, a brief *IGF-1R* inhibition in conjunction with continuous osimertinib treatment could result in the eradication of tumors and prevention of regrowth even after osimertinib discontinuation. These findings suggest that optimal inhibition of tolerant signals, when combined with osimertinib, has the potential to significantly enhance the outcome of EGFRmut-LC (23).

The results can be applied to transmission electron microscopy (TEM) and provide support for previous studies that explore the EMT epigenetic process (*Figure 2*). Baldavira *et al.* (24) explored organelles and EMT in NSCLC using TEM. They observed prominent organelles, such as the Golgi apparatus, mitochondria, and endoplasmic reticulum in malignant cells from adenocarcinoma, suggesting an activated hypersecretory state that is consistent with the protrusion and dissection of the basement membrane to invade the surrounding matrix. Additionally, small vesicles were observed in the cytoplasmic membrane of malignant cells (24).

Prieto *et al.* (25) also investigated the EMT process and organelles by genomic and ultrastructural analysis in neuroendocrine carcinomas. EMT transcription genes were found to be overexpressed, including *COL3A1*, *COL5A2*, and *SNAI2*, while the expression of *DSC2* was low. In addition, a lot of endosomal microvesicles and mitochondria were observed in the cytoplasm of atypical carcinoid and large cell neuroendocrine carcinoma. In small-cell carcinoma, the intercellular junctional complex was not prominent implying abnormal *DSC2* levels and loss of cohesivity leading to a fusiform transformation of malignant cells for invasion (25).

In summary, the exciting article by Tan *et al.* (11) unveils a novel dimension in understanding how the EMT triggers exocytic and endocytic vesicular trafficking programs in lung cancer. Although the study by Tan *et al.* (11) provides substantial insights into the functional role of *PI4K2A* and its targetability in lung cancer to surmount therapy resistance, additional experimental and clinical investigations conducted by diverse research groups are essential to corroborate the biological significance and

explore the potential clinical applications of these findings.

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.



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