Editorial

Small cell lung cancer heterogeneity: elevated a Notch above the *Rest*!

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Provenance: This is an invited Editorial commissioned by Section Editor Dr. Tianxiang Chen (Department of Thoracic Oncology, Shanghai Lung Cancer Center, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai, China).

Comment on: Lim JS, Ibaseta A, Fischer MM, *et al.* Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer. Nature 2017;545:360-4.

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The notch signaling pathway is an evolutionary conserved pathway that plays a central role in cell fate determination. In mammals, there are four Notch receptors and five ligands that can be either activating or inhibitory. To further add in complexity, Notch activity can either be canonical or non-canonical. The canonical signaling pathway is dependent on notch intracellular domain (NICD) nuclear translocation and interaction with *CBF-1/Suppressor of Hairless/LAG-1* (CSL) transcription factor (1). This leads to the activation of multiple genes such as hairy and enhancer of split-1 (Hes1), c-Myc, cyclinD1, and Akt (2). On the other-hand, the non-canonical pathway is independent of CSL interaction and could be independent of ligand interaction too (3).

In lung cancer, the Notch pathway is known to play either an oncogenic or a tumor suppressive role depending on histologic subtype. In lung adenocarcinoma, ample evidence of Notch upregulation and association with poor outcome supports an oncogenic role (4,5). While in small cell lung cancer (SCLC), 25% inactivating mutations in the Notch family were seen in human tumors and Notch activity was associated with less tumor formation and prolonged survival in SCLC mouse models (6). Notch signaling in SCLC can lead to cell cycle arrest, apoptosis, mesenchymal to epithelial transition, and suppression of neuroendocrine (NE) differentiation (7,8).

In Lim *et al.* manuscript, the cellular localization and Notch pathway activity was evaluated in a conditional triple knock out, $p53^{flox/flox}$;$Rb^{flox/flox}$;$p130^{flox/flox}$, SCLC mouse model, where green fluorescent protein (GFP) was expressed from the endogenous Hes1 promoter (9). Furthermore, data generated from this system were confirmed in cell lines and primary human SCLC tissues.

Lim *et al.* reported a differential cellular expression of Notch receptors and ligands as well as activity in SCLC tumors. The GFP^{high} representing Notch activity through the high expression of Hes1, had high Notch1/2/3 receptor and low NE genes expression. The GFP^{neg} cells indicating low Notch activity, expressed high level of Notch ligands and NE genes. In addition, the GFP^{high} cells were less proliferative and formed slower growing tumors than GFP^{neg} cells. Single cell qRT-PCR analysis showed that GFP^{high} cells express at least one Notch receptor but don't express Notch ligands (*Figure 1*). In contrast, the majority of GFP^{neg} cells express Notch ligands only; however, some GFP^{neg} cells do co-express Notch receptors too and are capable of expressing Hes1 when induced by a ligand. This dichotomy in receptor and ligand expression, favors an "inductive signaling" between distinct cell subpopulations. GFP^{high} and GFP^{neg} cells form a microenvironment where NE SCLC GFP^{neg} cells that express Notch ligands, induce Notch activation in the GFP^{high} adjoining cells. Notch signaling is a repressor of NE differentiation through decreased expression of NE-promoting transcription factors such as achaete-scute complex homologue 1 (ASCL1) (10). In this manuscript, *Rest* is identified as the transcriptional factor that suppresses the expression of neuronal genes,

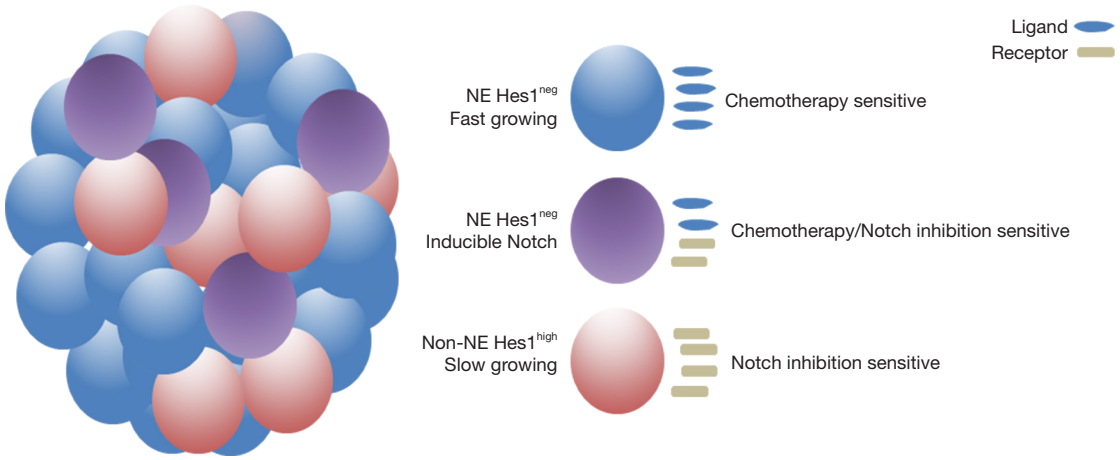

Figure 1 Modeling of SCLC tumors, adapted from Lim *et al.* Three types of cells are present with variable Notch signaling activity. Cells that have Notch receptors only are activated by ligands on neighboring cells and lose their NE features, becoming non-NE cells (pink). These non-NE SCLC cells promote the growth of NE cells that mostly harbor Notch ligands only (blue) except for a small subpopulation that can have both Notch receptors and ligand (purple) and are capable of inducing HES1. Combining chemotherapy or Notch ligands targeted therapy with Notch activity inhibition can manage both the NE cells and the non-NE cells. SCLC, small cell lung cancer; NE, neuroendocrine.

destining the GFP^{high} cells to a non-NE phenotype. In return, the GFP^{high} cells secret midkine that promotes the growth of GFP^{neg} NE SCLC cells. This unique intercellular Notch dependent communication is necessary for the development and progression of SCLC. Interestingly, once the differentiation status of the non-NE cells is attained through Notch activation, their cross talk and proliferation support of the GFP^{neg} cells is no longer dependent on Notch activity. This suggests that agents targeting Notch activity in GFP^{high} cells but lacking cytotoxic effect, might have limited success in controlling tumor growth. This is further complicated by the fact that GFP^{high} cells are more resistant to chemotherapy than GFP^{neg} cells and seem responsible for early relapse in mouse models. In this manuscript, targeting the GFP^{high} cells with Notch inhibitors in combination with chemotherapy, achieved a better tumor growth inhibition than either agent alone. The GFP^{high} cells had decreased proliferation and the GFP^{neg} NE cells had increased apoptosis.

This manuscript elegantly describes a self-sufficient system where the developing SCLC tumor is comprised of intra-tumoral heterogeneous cells forming a unique microenvironment to support survival and progression. Non-NE cells transformed by Notch activity, constitute about one fourth of the tumor mass, secrete growth factors to support NE cell proliferation, and are resistant to chemotherapy treatment. This system ensures the sustenance of the tumor and highlights the complexity of Notch activity in SCLC, where it acts as a tumor suppressor for the NE cells but an oncogene in the non-NE cells. Although the microenvironment generated by the intra-tumoral heterogeneity is important, it seems that other lung specific factors also play a role in Notch activity.

The knowledge provided in this manuscript underlines the challenges of treating SCLC. However, it also provides insights to potential reasons behind failure of Notch directed therapy in some clinical trials and the opportunity to design better treatment approaches. The rational for chemotherapy and Notch inhibition is based on strong molecular/cellular data which is validated validated in the mouse models. However, in clinical practice the combination of Tarextumab (Notch2/3 antibody) with chemotherapy, failed to improve progression free survival or overall survival in a phase 2 clinical trial (PINNACLE). Could this be explained by the authors findings that non-NE cells can still support the proliferation of the NE cells despite Notch inhibition? Is it possible that Notch1 receptor activity is capable of rescuing the Tarextumab Notch inhibition limited to Notch2,3 receptors; would a pan Notch antibody fair better? Is it worth re-probing gamma secretase inhibitors (GSI) at low doses that would be sufficient to inhibit non-NE SCLC without the toxicities associated with high intermittent dosing? Is a sequential Notch inhibition and chemotherapy regimen a valid

approach?

Moreover, the strategy of using Notch ligands as targets for antibody drug conjugates have shown some promising early results. A DLL3-targeted antibody-drug conjugate, Rovalpituzumab tesirine, had a confirmed 38% objective response, in patients with high DLL3 expression (11). Based on this manuscript, the NE GFP^{neg} would be the main target of Rovalpituzumab and thus a combination of Tarextumab (GFP^{high} cells) and Rovalpituzumab (GFP^{neg} cells) might represent a rational therapeutic combination.

This manuscript is a great example of the required dissection of pathways at the cellular and molecular levels to provide a better understanding of the pathophysiology of SCLC. It is only through this rigorous approach that meaningful strides would be made in altering the current clinical outcome seen with this recalcitrant cancer.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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