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INSIGHTS

Small cell lung cancers made from scratch

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In this issue of *JEM*, Chen et al. (https://doi.org/10.1084/jem.20181155) describe a new approach for the transformation of human pluripotent embryonic stem cells (hESCs) into neuroendocrine (NE) tumors of the lung closely resembling human small cell lung cancer (SCLC). Another recent study uses a different method to transform fully differentiated normal human cells into high-grade NE tumors (Park et al. 2018. *Science*. https://doi.org/10.1126/science.aat5749). These approaches and their models provide important new resources for developing diagnostic, preventative, and therapeutic approaches for high-grade NE tumors.

Small cell lung cancer (SCLC) is a deadly and highly metastatic form of lung cancer expressing a neuroendocrine (NE) program for which treatment has changed little over the past 30 years, causing the US Congress and National Cancer Institute (NCI) to label it a "recalcitrant" cancer (Gazdar et al., 2017). SCLC, when diagnosed, is nearly always widely metastatic, and while it has initial dramatic responses to chemotherapy and radiation therapy, it usually becomes resistant to these therapies, leading to patients' demise. Led by the NCI's "call to arms" to find new treatments for SCLC (under then NCI Director Harold Varmus), there is now a worldwide effort to identify new early detection, prevention, and therapeutic approaches. At the heart of this international effort is the development and use of preclinical models to test new strategies to facilitate clinical translation. Because of its metastatic nature, SCLC is usually histologically diagnosed by very small biopsy samples, meaning that for the vast majority of patients, there are scant tissue resources available for research studies, which makes the generation of renewable SCLC tumor resources paramount. This has been met by the worldwide sharing of a sizable panel of human SCLC cell lines and xenografts, including recently developed xenografts made directly from patient samples (patient-derived xenografts) and tumor cells circulating in the blood (Gazdar et al., 2017; Drapkin et al., 2018). In addition, a variety of genetically engineered mouse models (GEMMs) of SCLC have been developed (Gazdar et al., 2015). Nearly all of these human models (~200 combined) and ~100 SCLC patient tumor samples have undergone extensive molecular characterization to identify mutations, gene expression patterns, expression of "lineage oncogenes," and epigenomic changes found in SCLC (George et al., 2015). Taken together, these molecular studies show that SCLC is characterized by: mutational loss of the *TP53* and *RB1* tumor suppressor genes and expression of a NE mRNA program; being driven by lineage-specific transcription factors ("lineage oncogenes") such as ASCL1, NEUROD1, and POU2F3; overexpression of different members of the myc family (c-Myc, MYCL, or MYCN); and large numbers of other mutations and genetic alterations associated with cigarette smoking (George et al., 2015; Borromeo et al., 2016; Gazdar et al., 2017). In addition, ~10–15% of SCLCs express a "low NE phenotype" usually associated with lack of expression of ASCL1 or NEUROD1. By contrast, SCLC GEMMs generated by inactivation of TP53, RB1, and in some cases also p130 (RB2) elicited in many types of lung cells or targeted to selected NE pulmonary cells, express ASCL1 and various myc family members, but are almost devoid of other mutations (Gazdar et al., 2015). In human and GEMM SCLC studies, other key pathway changes recently found include NOTCH pathway inactivation and overexpression of NFIB in subclasses of the most metastatic SCLC models (George et al., 2015; Yang et al., 2018). Beginning with this information, Chen et al. made use of important new information and methodologies on how

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human embryonic stem cells (hESCs) can be made to differentiate into pulmonary precursors (Huang et al., 2015). In a technical tour de force, they treated hESCs with the pulmonary differentiation regimen to generate "pulmonary precursor" cells and then systematically explored the effect of inhibiting the Notch pathway pharmacologically (Shih and Wang, 2007) and genetically inactivating RB1 and TP53. Their key findings are that Notch inhibition led to an increase in cells with a pulmonary NE phenotype and that this NE program cell expansion was facilitated by inhibiting RB1 but not TP53. However, by the combined effect of inhibiting Notch, RB1, and TP53 over several months, they had best expansion of pulmonary NE cells, and these expanded altered cells were capable of forming tumors in immunodeprived mice with characteristics of SCLC. By contrast, genetically altering these same precursor cells with mutant KRAS or mutant EGFR (alterations that are found in lung adenocarcinomas) did not lead to the expansion of pulmonary NE cells or SCLC development. This work thus provides entirely new

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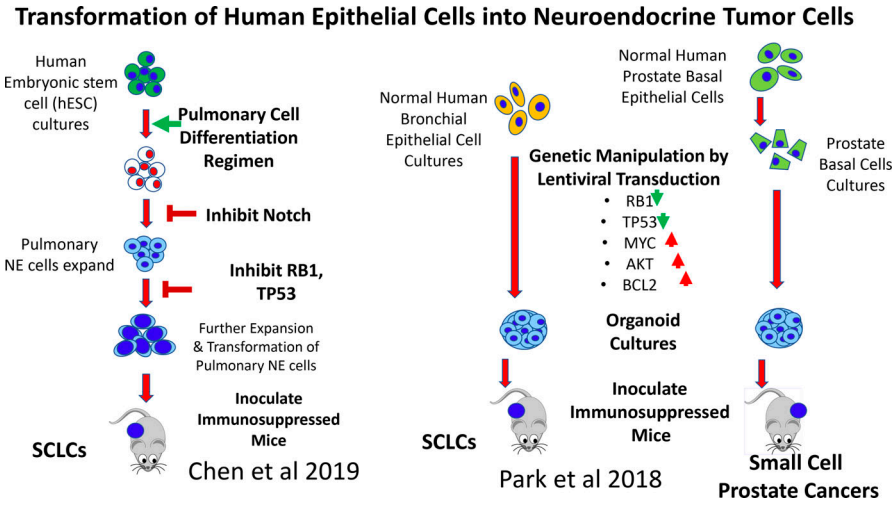
preclinical models to study the pathogenesis and explore new therapeutics for SCLC.

The end result of the study by Chen et al. (2019) was the generation of malignant NE cells which closely resembled SCLC in xenografts and expressed major SCLC properties including NE genes, the transcriptional factor *NKX2-1*, MYC family genes, and overexpression of proliferative and antiapoptotic genes, two of the hallmarks of cancer (Hanahan and Weinberg, 2011). One of the important results of this work was the demonstration of how this approach could be used to identify the roles of different genetic changes in NE tumor pathogenesis. For example, the authors found that inhibition of *RB1* in conjunction with inhibition of *NOTCH* resulted in a NE phenotype, while inhibition of *TP53* resulted in a hyperproliferative, antiapoptotic phenotype. Overall, this study showed the power of integrating recently developed cellular and molecular biology technologies (lineage differentiation protocols, controlled genetic and pharmacologic manipulation, and single-cell RNA sequencing [scRNA-seq]) for studying cancer lineage pathogenesis. As in all important reports, Chen et al. (2019) have opened up many other things to study. For example, while Notch inhibition in hESC pulmonary precursors led to ∼10% turning into pulmonary NE cells (PNECs), this raises important questions: What made these 10% susceptible to PNEC conversion, what occurred in the other 90%, and could there be pharmacologic and genetic screens to determine other ways to convert hESCs pulmonary precursors into PNECs? Also, the roles of lineage transcription factors ASCL1, NEUROD1, myc family members, and the recently described Nfib transcription factor, both in NE differentiation and in SCLC tumorigenesis, remain open questions. One puzzle was why many of the malignant cells expressed *NEUROD1* as their neural lineage-specific transcription factor rather than *ASCL1*. *NEUROD1*, a potent neural transcription factor, plays a secondary role to the more commonly expressed *ASCL1* in SCLC, and its role or appearance in cancer pathogenesis is not fully understood. By contrast to *ASCL1*, *NEUROD1* is not expressed in the bronchial epithelium, although it is present in the ganglion cells of the lung, and its knockout does not prevent tumor formation in a GEMM model of SCLC, while *ASCL1* knockout does (Borromeo et al., 2016). In a *myc*-driven GEMM of SCLC (Mollaoglu et al., 2017), the early preinvasive and invasive lesions express *ascl1*, followed by the rapid transition to *neurod1* expressing cells with partial loss of NE differentiation. While *MYC* family genes are often overexpressed in the current transformation model, the relationship between *MYC* and *NEUROD1* is not simple or fully understood. In addition, the detailed scRNA-seq data provided by the authors indicate in nearly every case multiple subpopulations of cells within the different manipulation groups (Notch inhibition, TP53 or RB1 inactivation), indicating that there is a very rich "tapestry" of biology still to explore. Likewise, what are the lineage differences that make pulmonary NE cells not respond to potent oncogenes like mutant KRAS and EGFR?

NE tumors morphologically resembling SCLC may arise in many different organs (Ochsenreither et al., 2009). The best studied of these are NE tumors arising in the prostate, often by transformation of adenocarcinomas to NE tumors after the development of androgen resistance, a process known as transdifferentiation (Nadal et al., 2014). Thus, it was relevant to compare the current work to a recent report (Park et al., 2018) that used fully differentiated normal human bronchial epithelial cells or prostate basal cells (the precursor cells of prostatic epithelium) and transformed them into NE tumor cells of lung or prostate origin, using a combination of five genetic changes ("PARCB") for TP53 dominant negative, myristoylated AKT (PTEN inhibition), RB1 knockdown, and c-Myc and Bcl2 overexpression. This comparison indicates that the Chen et al. (2019) approach could be extended to other lineages, and also shows the power of the hESC approach in dissecting the earliest steps in pathogenesis. Essentially all of the PARCB changes were required to get SCLC development in the Park model, while we know that only subsets of human SCLCs overexpress c-Myc and Bcl2 and inactivate PTEN. In any event, both approaches represent important steps forward.

Current therapeutic strategies designed to inhibit oncogenic pathways driving malignant phenotypes frequently result in treatment-resistant cancers. SCLC is characterized by its initial responses to therapy followed almost inevitably by the development of resistance to further cytotoxic therapies. These new approaches offer new clinical translational opportunities. For example, we urgently need markers for the



Two models for the transformation of normal human cells to NE tumors are outlined. Chen et al. (2019) started with cultures of hESCs, converted them into pulmonary NE cells, and then sequentially inactivated the Notch pathway, *RB1* and *TP53*. The resultant cells and tumors derived from the inoculation of the cells into immunosuppressed mice closely resembled SCLC cells and tumors respectively, both morphologically and genetically. Park et al. (2018) started with cultures derived from either human bronchial epithelial cells or adult prostate tissues. From the prostate cells, they isolated basal cells, the precursors of the prostatic epithelium. They used lentiviral transduction to perform five genetic manipulations, inhibition of *RB1* and *TP53*, and up-regulation of *MYC, AKT*, and *BCL2*. The transduced cells were grown as organoid cultures and subsequently inoculated into immunosuppressed mice. The resultant tumors closely resembled either SCLC (from bronchial epithelial cells) or small cell prostate cancers (from prostate cultures), both morphologically and genetically.

very early detection of SCLC in a "preneoplastic" phase, and the scRNA-seq data from the current Chen et al. (2019) study provide both data and approaches to generating such markers. We need new ways to screen for chemoprevention agents for SCLC and the Chen model provides reagents for such screens. The Chen et al. (2019) tumors were not metastatic, so their model provides a way to systematically test for genetic changes associated with the development of metastases and Nfib overexpression is an excellent first candidate. Recently, we learned that GEMM SCLCs that arise from different pulmonary stem cells can have many of the same characteristics but behave biologically quite differently (Yang et al., 2018). The Chen model will allow study of these aspects in human cells. Does resistance to platin-etoposide (seen every day in the clinic) occur in these models, or does it require a more complex genetic background? Conversely, do new therapies identified in drug screens of patient-derived SCLC preclinical models (with many other genetic changes) have activity in the hESC SCLC models with their very simple genetic alterations? Surprisingly, aggressive NE tumors arising in several organs may share clinical characteristics and molecular features of both stem-like and NE lineages induced by shared genetic and epigenetic processes (Smith et al., 2018). These findings suggest that aggressive NE tumors arising in diverse organs may be amenable to common therapeutic modalities. The Chen and Park models represent the transformation of stem cells or normal cells, recapitulating the multistep pathogenesis of two common (lung and prostate) forms of NE tumors. Similar procedures may result in the generation of still further types of NE tumors. They represent important models to study sequential development of, and provide important clues for, new forms of targeted therapies. The common features of NE tumors arising at multiple sites offer the promise that similar therapeutic approaches could apply to NE tumors arising at these sites. Finally, it will be important to combine these "cell autonomous" models of NE cancer with components of the tumor microenvironment to determine these important inter-relationships.

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