Treating Transcriptional Addiction in Small Cell Lung Cancer

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Small cell lung cancer (SCLC) is a devastating tumor type with great therapeutic need. In this issue of *Cancer Cell*, Christensen and colleagues identify THZ1, a CDK7 inhibitor, as a potential therapy for SCLC. Using cells and mouse models, the authors show exquisite sensitivity of SCLC to transcriptional inhibition.

Small cell lung cancer (SCLC) is a neuroendocrine tumor type that represents ~15% of lung cancer diagnoses. SCLC is typically metastatic when diagnosed and is considered the deadliest lung cancer subtype with an overall 5-year survival of less than 5%. Although SCLC is typically very responsive to chemotherapy, the response is short lived, and chemorefractory SCLC almost invariably emerges. In contrast to non-small cell lung cancer (NSCLC), for which a number of targeted therapies are available, SCLC has not seen substantial improvements in therapies over the past four decades, and no approved targeted therapies exist for SCLC. Next-generation sequencing analyses highlight a complex genomic landscape in SCLC with high numbers of protein-altering mutations (Peifer et al., 2012; Rudin et al., 2012). However, low numbers of SCLC samples have been sequenced to date relative to other major cancers, and clear SCLC-mutated drug targets have not yet emerged. With the focus of the translational lung cancer research community almost entirely on NSCLC, targeted therapies for SCLC have lagged behind the need.

To address the need for improved SCLC therapies, Christensen et al. (2014 in this issue of *Cancer Cell*) screened over 1,000 experimental and clinical compounds for efficacy across three murine SCLC (mSCLC) cell lines. The cell lines were isolated from a genetically engineered mouse (GEM) model for SCLC that is based on deletion of *Rb* and *p53* in the lung epithelium; this model recapitulates the key features of human SCLC (Meuwissen et al., 2003). Of screen hits that included cell cycle inhibitors, mTOR-PI3-kinase pathway inhibitors, and transcriptional inhibitors, the authors focused their attention on THZ1, a transcriptional inhibitor that acts by forming a covalent interaction with CDK7. The authors found that SCLC lines were ~5-fold more sensitive to THZ1 growth inhibition than NSCLC cell lines.

CDK7 regulates transcription initiation by phosphorylating the C-terminal domain of RNA polymerase II. CDK7 is also a component of the CDK activating kinase that controls activation of the cell cycle by driving cyclin-dependent kinases, including CDK1 and CDK2. THZ1 forms a covalent link to a cysteine residue located outside the CDK7 canonical kinase domain to irreversibly inactivate CDK7 (Kwiatkowski et al., 2014). A previous study found that THZ1 treatment led to potent antiproliferative effects in T cell acute lymphoblastic leukemia cells and xenografts (Kwiatkowski et al., 2014).

Christensen et al. (2014) followed up on their observations of THZ1 sensitivity in SCLC cell lines to investigate THZ1 efficacy in vivo using the autochthonous SCLC GEM model (Meuwissen et al., 2003). Using MRI to image SCLC tumor volume at baseline and following treatment, they report that THZ1 resulted in reduced tumor progression and, in some cases, dramatic tumor regression. THZ1 treatment also extended survival of animals with mSCLC. THZ1 treatment showed in vivo effects in the model comparable to that found for the standard chemotherapeutic regimen (cisplatin/etoposide). Finding a targeted therapeutic that could be added to a cisplatin/etoposide chemotherapy regimen might harness the chemosensitivity seen in human SCLC and lead to a durable patient response. Unfortunately, combining THZ1 with cisplatin/etoposide did not result in a stronger tumor regression than either drug regimen alone. However, THZ1 was effective in xenograft models that were generated from chemorefractory cell lines, suggesting potential for efficacy in human chemorefractory SCLC. Importantly, in contrast to cisplatin/etoposide treatment in mice, THZ1 treatment was not associated with detectable toxicity.

The authors next investigated the underlining mechanisms associated with THZ1 sensitivity in SCLC. Among the top differentially expressed transcripts upon THZ1 treatment in SCLC cell lines were genes associated with transcription. In previous work from this group, THZ1 preferentially reduced the expression of genes associated with a subtype of transcriptional enhancers termed "super-enhancers" (Kwiatkowski et al., 2014). Typical enhancers are composed of transcription factor binding sites located at a distance from the transcriptional start site that act through chromosomal looping events to enhance transcription. Super-enhancers consist of very large clusters of enhancers and have been associated with highly expressed genes that confer cell identity (Whyte et al., 2013), and, in cancer cells, include oncogenes (Lovén et al., 2013).

The authors mapped enhancers and super-enhancers in three human SCLC lines by performing ChIP-seq analyses against acetylated lysine 27 of histone H3. An average of ~100 super-enhancer associated genes were identified in the SCLC cell lines, including genes encoding oncogenic transcription factors such as MYC family members (*MYC* and *MYCN*), *SOX2*, and *NFIB*. Genes encoding lineage transcription factors such as *INSM1*, *ASCL1*, and *NEUROD1* were also identified as harboring super-enhancers or

atypically large enhancers in SCLC. Moreover, transcripts reduced upon THZ1 treatment were enriched for those whose genes contain super-enhancers, including MYC members, NFIB, and lineage transcription factors. Thus, candidate mediators of THZ1 response in SCLC include oncogenic and neuroendocrine lineage transcription factors.

Christensen et al. (2014) have identified a promising candidate drug for potential clinical use with strong antiproliferative effects in both chemo-naive and chemorefractory SCLC. It is not yet clear whether subsets of SCLC patients may preferentially benefit from treatment with THZ1 or other transcriptional inhibitors. In cell culture, THZ1 was widely effective across SCLC cell lines regardless of what specific genes were mutated, but, in vivo, THZ1 treatment resulted in strikingly different responses in different animals. Three of nine THZ1-treated mice had remarkable responses to THZ1 treatment while other animals showed little effect or exhibited stable disease without tumor regression. One major question that results from the work is: what confers sensitivity of SCLC to THZ1 treatment in vivo? SCLC in the mouse model is simpler genetically than human SCLC but still exhibits spontaneous and heterogeneous secondary alterations, such as high level *Mycl1* or *Nfib* gene amplifications (McFadden et al., 2014). It would be interesting for future studies to link secondary alterations that occur in the model to THZ1 response in vivo. THZ1 was recently shown to be particularly effective in the context of *MYCN*-amplified neuroblastoma (Chipumuro et al., 2014) and may be broadly effective in MYC family amplified tumors. *MYCL1* is the most frequently amplified MYC member in SCLC, and it would be interesting to determine whether a more homogeneous in vivo response to THZ1 might be obtained in an SCLC mouse model driven by *Mycl1* overexpression (Huijbers et al., 2014). Christensen et al.'s work also draws attention to genes encoding neural/neuroendocrine lineage transcription factors, such as *ASCL1*, *NEUROD1,* and *INSM1* that were sensitive to transcriptional inhibition using THZ1. Roles for such factors in THZ1 response need to be explored, because transcription factors controlling neuroendocrine cell state may themselves reflect therapeutic vulnerabilities in SCLC.

The identification of effective therapies for SCLC remains a major challenge. However, this work opens up a novel avenue in the exploration of transcriptional inhibitors as potential new treatments for SCLC.

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