CANCER

Small cell lung cancer: Time to revisit DNA-damaging chemotherapy

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Rational use of DNA-damaging chemotherapy, with new combinations to heighten DNA replication stress, could improve outcomes in small cell lung cancer.

Lung cancer is the leading cause of cancer deaths worldwide. Small cell lung cancer (SCLC), caused largely by tobacco smoke, is the most aggressive type of lung cancer. It represents 15% of all lung cancers, with an annual incidence of more than 34,000 in the United States alone. Chemotherapy with or without radiation is the standard treatment. Recent treatment efforts have been largely focused on molecularly targeted agents and immune-mediated therapies; although blocking immune inhibitory pathways remains an area of active investigation, targeting oncogenic drivers has mostly proven disappointing. As a result, chemotherapy remains the backbone of treatment. The combination of cisplatin or carboplatin with etoposide or irinotecan is the commonly used initial regimen for patients with advanced SCLC. Although initial response rates to chemotherapy are very high, the cancer usually relapses within months, and nearly all cases relapse within 1 year, becoming generally unresponsive to additional chemotherapy, with fewer than 5% of patients surviving 2 years. The only approved drug for relapsed disease is topotecan. Other drugs with more modest activity include amrubicin, doxorubicin, paclitaxel, and temozolomide.

Active chemotherapeutic agents in SCLC have one thing in common: almost all of them—except paclitaxel, which disrupts microtubule function—interfere with DNA replication. Cisplatin and carboplatin form cross-links between DNA bases, creating DNA replication barriers. Topotecan, irinotecan, etoposide, doxorubicin, and amrubicin trap topoisomerases, stalling replication forks and causing toxic double-stranded DNA breaks.

Recent advances in drug discovery and genomics are offering unprecedented opportunities to target molecular defects that underlie cancer. Accumulating evidence suggests that we may be at the cusp of uncovering ways to rationally apply DNA-damaging chemotherapy in SCLC. Here, we define the hallmarks of SCLC; highlight DNA replication stress, a key consequence of these hallmarks; and then discuss ways to take advantage of replication stress using DNA repair and checkpoint-targeted drugs.

HALLMARKS OF SCLC

The hallmarks of cancer are biological capabilities acquired during tumorigenesis that enable tumor growth and metastatic dissemination (*1*). For a specific cancer, some hallmarks are more crucial than others. As evidenced by recurrent genetic alterations, the following hallmarks emerge as central to SCLC: sustained proliferation, resistance to apoptosis, unlimited replicative potential, genomic instability, and evasion of growth suppressors (Fig. 1).

The tumor suppressors *RB1* and *TP53* are lost in nearly 90% of SCLC (*2*), and inactivation of *Rb1* and *Tp53* in mouse models induces tumors that closely resemble human SCLC. Inactivation of *RB1* plays a central role in proliferation by allowing cells to enter the cell cycle. In addition, transcriptional up-regulation and amplification of the *MYC* family of proto-oncogenes and others such as *SOX2* are found in most SCLC cell lines and drive the expression of genes that sustain cell proliferation (*3*). These genes are key drivers for the maintenance of pluripotency and self-renewal of cancer stem-like cells. Persistence of a subpopulation of these cells during chemotherapy contributes to the rapid emergence of drug-resistant clones (*4*).

TP53 inactivation, which is much more prevalent in SCLC than previously thought (*2*), prevents cell cycle arrest and apoptosis. Elevated expression of the antiapoptotic protein BCL2 is found in most SCLC, further establishing resistance to apoptosis as a relevant hallmark. Histone modifications, although not linked to a specific hallmark, regulate multiple hallmarks by modulating key cellular processes such as gene transcription, DNA replication, and DNA repair; recurrent mutations in chromatin modifiers are found in a subset of SCLC.

REPLICATION STRESS IS A SOURCE OF GENOMIC INSTABILITY IN SCLC

SCLC chromosomes and genomes are highly abnormal, and genomic instability in SCLC is among the highest in all cancers. Although alterations in DNA repair genes have not been systematically explored, poly[adenosine diphosphate (ADP)–ribose] polymerase 1 (PARP1), a DNA repair protein, tends to be highly expressed in SCLC (*5*), as are genes involved in homologous recombination (HR) and DNA replication, suggesting that SCLC may require HR and PARP1 for unchecked proliferation.

Replication is a concerted process involving fast-moving replication forks, adequate deoxynucleotide pools, and a proper DNA template (*6*). Any disturbance in these coordinated processes causes replication stress and collapse of DNA replication forks. In the context of SCLC, replication stress is primarily driven by rapid proliferation caused by activation of oncogenes such as *MYC* in the absence of tumor suppressors such as *RB1* and *TP53*. In addition, the many genomic alterations in SCLC (Fig. 1) are also likely to produce replication blocks when the replicative polymerases attempt to copy abnormal templates or when origins of replication fire at inappropriate sites and times, making SCLC dependent on DNA repair and cell cycle checkpoints for growth.

EMERGING STRATEGIES TO PRECIPITATE SCLC REPLICATION STRESS

Replicative stress is pervasive in SCLC, and therapeutic approaches that exacerbate this stress could selectively kill SCLC by replicative damage. Currently used DNA-damaging chemotherapies already generate replication stress, and we posit that combinations of DNA-damaging chemotherapy with drugs targeting mediators of cellular responses to replication stress are rational therapeutic avenues for SCLC (Fig. 1). Thus, clinical detection of replication stress using biomarkers such as phosphorylated histone H2AX, accumulation of single-stranded DNA, and others is essential to foster the development of therapies that exacerbate replication stress.

One emerging approach to exacerbate replication stress is the use of ataxia telangiectasia and Rad3-related kinase (ATR) inhibitors. ATR is required to stabilize replication forks, tune down replication speed, and promote HR and DNA repair, which are critical in oncogene-driven tumors. ATR inhibition is

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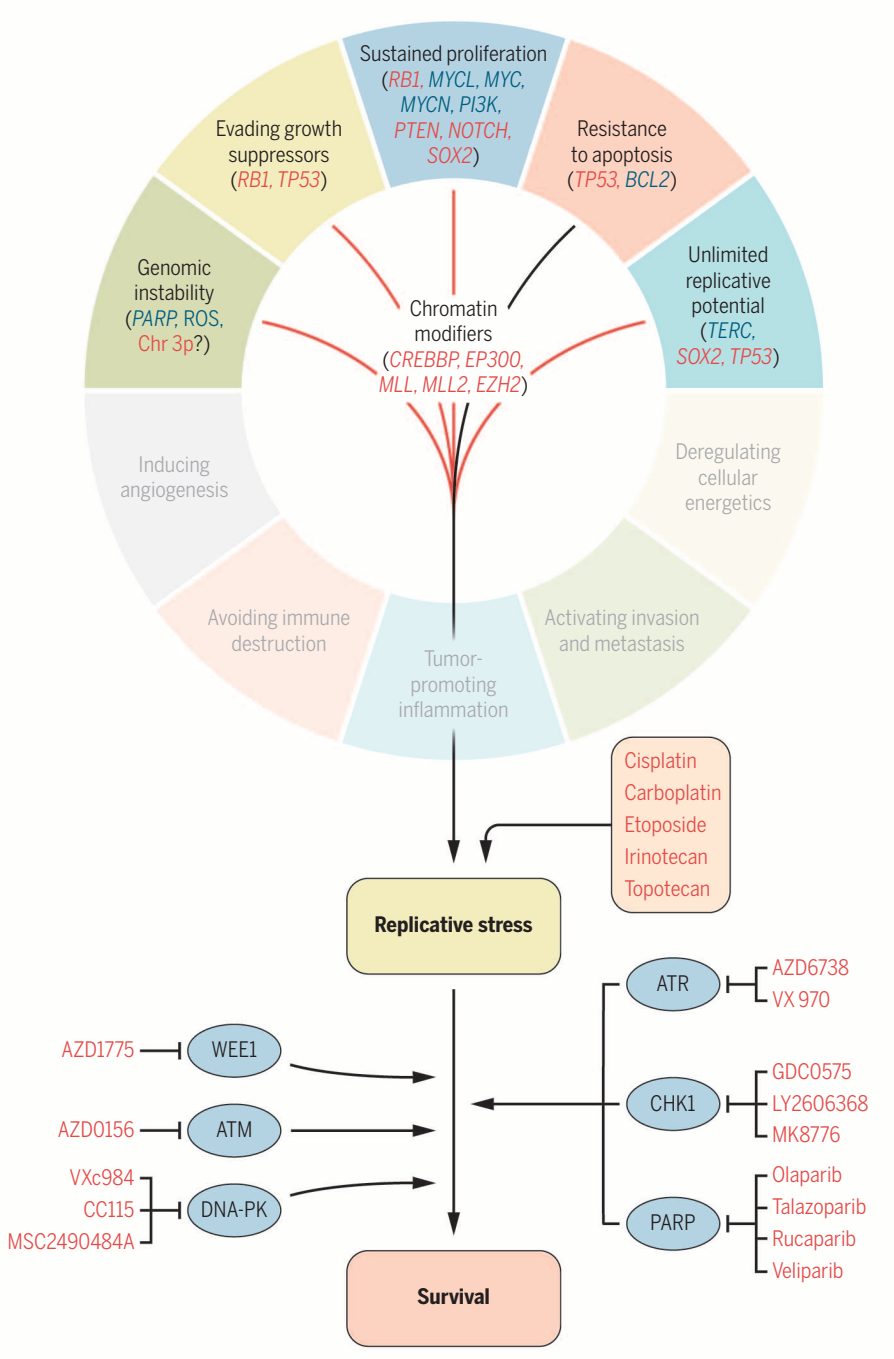

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Fig. 1. Replication stress in SCLC. The hallmarks of SCLC include sustained proliferation, resistance to apoptosis, unlimited replicative potential, genomic instability, and evasion of growth suppressors (genes that are up-regulated are indicated in blue and those that are down-regulated in red). All of these hallmarks except resistance to apoptosis have been directly linked to replicative stress. Therapeutic approaches that further exacerbate replication stress by inhibiting the target proteins shown in the figure could selectively kill SCLC by replicative damage. The hallmarks in the bottom half of the circle, in lighter colors, are not discussed in this article. Figure modified from (*1*). ATM, ataxia-telangiectasia mutated; DNA-PK, DNA-dependent protein kinase; ROS, reactive oxygen species.

particularly toxic for p53-deficient tumor cells, and this effect is exacerbated by replication stress–inducing states such as cyclin E overexpression and treatment with topoisomerase inhibitors (*7*, *8*). Although plagued by initial concerns regarding the essential nature of ATR function and paucity of selective drugs, inhibitors VX970 and AZD6738 are now in clinical trials.

CHK1 inhibitors are highly effective in killing replication stress–driven tumors. Upon replicative stress, CHK1 is required to effect cell cycle arrest downstream from ATR. Early attempts to target CHK1 with UCN-01 were unsuccessful, but a newer generation of selective CHK1 inhibitors (Fig. 1) has shown promise in preclinical studies and is being evaluated in clinical trials. A newcomer to the cell cycle checkpoint armamentarium is the WEE1 inhibitor AZD1775. WEE1 limits the activation of cyclin-dependent kinases; WEE1 inhibition induces replication stress by promoting aberrant firing of replication origins and subsequent nucleotide shortage. In a phase 1 trial, AZD1775 resulted in some partial responses in different cancer types (*9*).

Stalled replication forks activate PARP1, which catalyzes PARylation, a posttranslational modification in which ADP-ribose units are attached to target proteins. Recent studies indicate that PARylation of replication helicases and chromatin is critical for restarting replication forks upon DNA damage. PARP inhibition, by destabilizing replication forks, could provide a useful strategy to further enhance replication stress in SCLC. Clinical PARP inhibitors differ with respect to their catalytic inhibitory properties and relative ability to trap PARP enzymes on DNA, which is an important consideration when combining them with DNA-damaging chemotherapy.

Emerging evidence suggests that combinations of DNA-damaging chemotherapy and drugs that promote replication stress would further boost replication stress in a catastrophic manner and could be therapeutically used to drive SCLC responses. The ATR inhibitor VX970 markedly sensitized lung cancer cell lines and primary tumors to multiple DNA-damaging chemotherapies but was most effective in combination with topoisomerase I inhibitors. On the other hand, CHK1 inhibitors are more effective in combination with antimetabolites, indicating that despite acting on the same signaling cascade, ATR and CHK1 inhibition have distinct consequences. Inhibition of WEE1 and PARP is also highly effective in combination with DNA-damaging chemotherapy in tumors with and without defects in HR.

TARGETED DELIVERY OF DNA-DAMAGING CHEMOTHERAPY

The most challenging aspect of combinations involving DNA-damaging chemotherapy is

the acute and long-term toxicities to nontumor tissue that can affect virtually every organ of the body. Efforts to design less toxic DNA-damaging chemotherapy initially focused on developing analogs with chemical modifications. Although this approach yielded some early successes such as carboplatin, the less nephrotoxic derivative of cisplatin, the drugs in general provided only modest improvements in tolerability and efficacy. Ongoing efforts are focused on achieving targeted drug delivery and retention while minimizing drug accumulation in normal tissues.

Antibody drug conjugates (ADCs), monoclonal antibodies targeting tumor-specific proteins conjugated to chemotherapy, effectively increase the therapeutic index of chemotherapy. Although the early ADCs on the market were designed with tubulin inhibitors, an increasing number of ADCs in development use DNA-damaging compounds. Preliminary reports on these agents indicate strikingly less toxicity than conventional DNA-damaging chemotherapy. Another approach to targeted delivery involves the use of polymeric nanoparticles or liposomes, which are designed to increase drug concentration at tumor sites relative to healthy tissue. A liposomal irinotecan, Onivyde, was recently approved for patients with metastatic pancreatic adenocarcinoma, and others, including CRLX101, a nanoparticle camptothecin, are in clinical trials. Tumor selectivity of nanoparticle-based DNA-damaging agents can be further enhanced by decorating the nanoparticle with tumor-targeting ligands.

CONCLUSION

SCLC is the most lethal type of lung cancer and is among the most recalcitrant cancers. The majority of patients present with metastatic disease, where systemic therapy is the only treatment option. Given the growing number of drugs targeting genomic stress and of cytotoxic conjugates with tumor-targeted delivery, it is time to fully capitalize on the gains from DNA-damaging chemotherapy. As described here, replicative stress is a common feature of SCLC, driven by its many hallmarks. Recent studies also point to heightened replication stress in pluripotent stem cells that drive genomic instability and continued cancer progression (*10*). Although it is not known whether these observations extend to SCLC, it is plausible that targeting replicative stress could forestall the rapid emergence of resistance resulting from persistent stem cells. Heightening replication stress in SCLC using DNA-damaging chemotherapy can push SCLC beyond its survival threshold. The importance of serial biopsies to examine the molecular basis of sensitivity and resistance to drug combinations such as those discussed here cannot be overemphasized. It is heartening that a number of groups, most notably the one led by the U.S. National Cancer Institute (NCI) under a congressional mandate (H.R. 733, The Recalcitrant Cancer Research Act of 2012), are actively addressing this issue.

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