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Awakening of "Schlafen11" to Tackle Chemotherapy Resistance in SCLC

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http://dx.doi.org/10.1016/j.ccell.2017.01.013

Chemotherapy resistance arises invariably in small cell lung cancer (SCLC). In this issue of *Cancer Cell*, Gardner et al. find that in some SCLC, EZH2 mediates resistance via downregulation of Schlafen11 (*SLFN11*). Combining EZH2 inhibition with chemotherapy effectively overcomes drug resistance of xenografted SCLC, holding promise for new treatment paradigms.

Small cell lung cancer (SCLC), a cancer type almost exclusively observed in heavy smokers, is among the most devastating tumor types. Hardly any treatment advances have been made over the last 30 years, and most patients succumb to the disease within a year. The first-line treatment is either cisplatin or carboplatin in combination with the topoisomerase II inhibitor etoposide. Although many SCLC patients respond quite well initially, they invariably and rapidly relapse with drug refractory disease, leading to their dismal prognosis. As a second line of treatment, the topoisomerase I inhibitor irinotecan is standard but provides only very limited life extension. Evidently, new treatment modalities are urgently needed. While there is much interest in immunotherapy approaches to target SCLC, especially since it has one of the highest mutation loads, successes in SCLC are still modest with no evident benefit of ipilimumab added to the standard regimen (Reck et al., 2016). The observation that SCLC patients often respond well initially to chemotherapy but quickly develop refractory disease provides an opportunity to understand the nature of acquired chemotherapy resistance, which in turn might provide new inroads for better treatment.

This is addressed in the article by Gardner et al. (2017). The authors exposed mouse xenografts of chemonaive human tumors to sequential cycles of transplantation and cisplatin and etoposide (C/E) regimens. This resulted in matched pairs of chemonaive and chemoresistant tumors that retained these features over time (ten in total). DNA sequencing was performed on the matched pairs, but no recurrent changes were found in specific genes, nor did copy number alterations point to genes responsible for the acquired resistance. As a next step, they performed RNA-seq and observed that expression patterns between the chemonaive and resistant pairs were remarkably similar but did show distinct recurrent expression differences. Four out of ten of the chemoresistant tumors showed downregulation of Schlafen11 (*SLFN11*), a gene whose expression has previously been associated with sensitivity to DNA damaging drugs in various cancers (Barretina et al., 2012). Another chemoresistant subset showed upregulation of the transcription factor *TWIST1*, a gene associated with epithelial-mesenchymal transition and also drug resistance. In subsequent attempts to establish a causal relationship between the expression of these genes and chemoresistance, the authors found that *TWIST1* expression is not directly implicated in acquired resistance. In contrast, overexpression of *SLFN11* in chemoresistant human cell lines did largely restore sensitivity to topoisomerase poisons, indicating that *SLFN11* expression directly contributes to chemosensitivity. In accordance with these observations, SCLC cell lines from chemotherapy-treated patients exhibited overall lower *SLFN11* levels than from untreated patients.

Since downregulation of *SLFN11* has recently been connected to epigenetic silencing (Nogales et al., 2016), EZH2 is often highly expressed in SCLC, and EZH2 binding sites were found upstream of *SLFN11*, the authors investigated whether EZH2 plays a role in regulating *SLFN11*. A week-long exposure of cells ex vivo to either 5-AzaC or the EZH2 inhibitor EPZ011989 (EPZ) showed a striking increase in *SLFN11* expression only in EPZ-treated cells, indicating that histone H3K27 trimethylation is likely responsible for the downregulation of *SLFN11*. Treatment with EPZ also partly restored chemosensitivity in ex vivo PDX

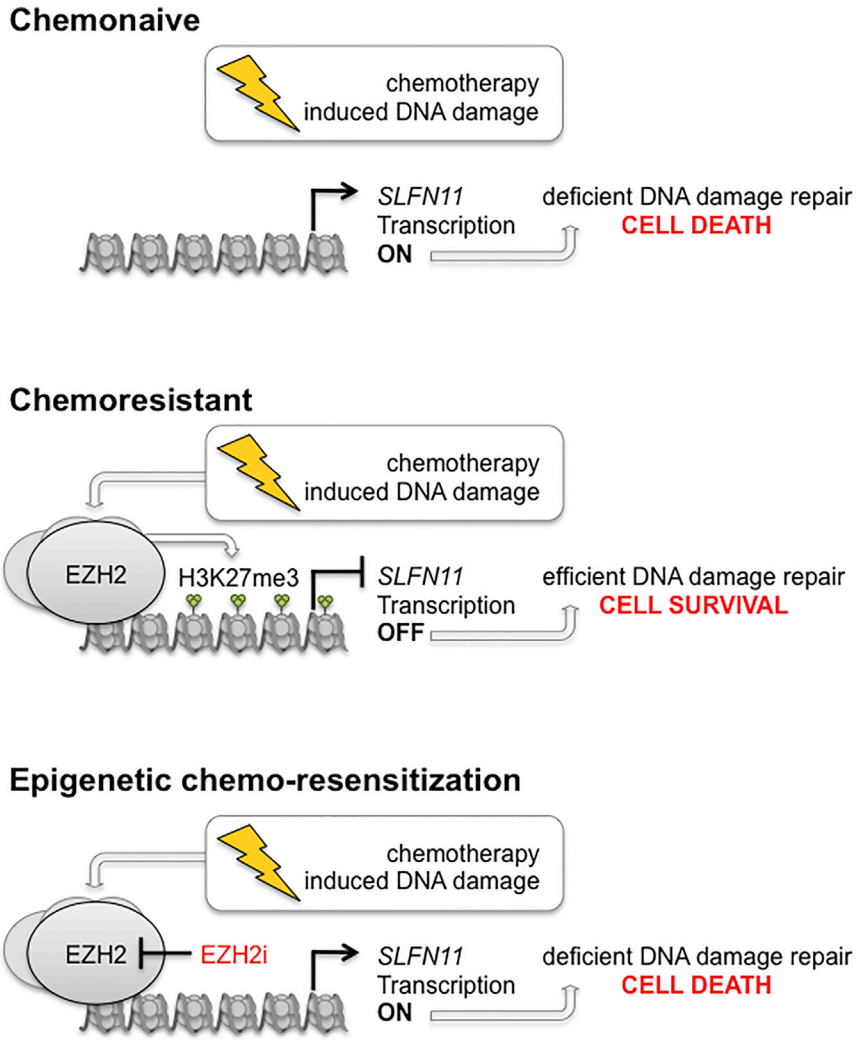

Figure 1. Overcoming Chemotherapy Resistance by Reversing EZH2-mediated Downregulation of *SLFN11*

Upper panel: chemonaive tumors express *SLFN11*, compromising their DNA repair activity and therefore responding to a combination of cisplatin and topoisomerase inhibitors. Middle panel: EZH2 is induced upon cytotoxic chemotherapy, leading to the deposition of H3K27me3 repressive chromatin marks in the *SLFN11* gene body, resulting in *SLFN11* repression. This leads to more effective DNA repair allowing cells to survive. Lower panel: *SLFN11* is re-expressed upon inhibiting EZH2 with concomitant restoration of sensitivity to cisplatin and topoisomerase inhibitors.

lines and in cell lines isolated from treated patients. This EPZ-mediated sensitization could be abolished by the concomitant downregulation of *SLFN11* through shRNA inhibition, indicating that SLFN11 is instrumental for the chemosensitivity.

Utilizing their in vivo xenograft models, the authors subsequently showed that treatment with EPZ was well tolerated and as a single agent resulted in a modest reduction in tumor growth. While H3K27me3 deposition at the transcription start site (TSS) was largely retained, EPZ exposure resulted in erasure of H3K27me3 throughout the *SLFN11* gene body with a concomitant increase in H3K27Ac both near the TSS and in the gene body. Interestingly, tumors that showed undetectable *SLFN11* levels at baseline did not start to re-express *SLFN11* upon EPZ treatment, suggesting alternative routes can repress *SLFN11* expression.

These observations led the authors to further explore EZH2 inhibition as an add-on to either first-line (C/E) or second-line (irinotecan) treatment in those tumors in which expression of *SLFN11* was lost upon acquiring chemoresistance. In both the first as well as the second line setting, addition of EPZ had pronounced effects on these xenografts. Combining EPZ with C/E could fully ablate tumors in the chemonaive setting while restoring sensitivity of chemoresistant tumors to irinotecan. Therefore, the combination of EZH2 inhibition and chemotherapy could be a particularly promising combination for further exploration in SCLC patients that express *SLFN11* and initially respond to C/E (Figure 1).

Whereas specific mutations that mediate acquired resistance to targeted therapies and more recently immunotherapy (Zaretsky et al., 2016) were found easily, identifying mechanisms of acquired chemotherapy resistance has proven more cumbersome. Changes in drug transport, drug detoxification and effective DNA repair appear obvious routes of resistance, but these changes might not be readily achieved by specific mutations as they may require multi-gene adaptations. As such, epigenetic reprogramming may serve as a more versatile approach for cancer cells to achieve the changes required for chemoresistance. Although SLFN11 depletion appears critically involved in drug resistance in a subset of SCLC, EZH2 inhibition may, in addition to restoring *SLFN11* expression, affect other genes that contribute to acquired resistance. This is in line with the more modest effects seen upon restoration of *SLFN11* expression alone as compared to its re-expression through EZH2 inhibition. Obviously, one would like to know the nature of the additional genes induced by EZH2 inhibition. Furthermore, it will be of interest to know how cells actually benefit from *SLFN11* expression in the first place. The reported activity of *SLFN11* in impairing checkpoint maintenance and homologous recombination repair by destabilizing RPA-ssDNA complexes (Mu et al., 2016) indicates that it may facilitate response to replication stress. However, since not all tumors are SLFN11 positive, its expression is apparently not under strong positive selection. The need to better understand its mechanisms of action is also inspired by recent work showing that loss of *SLFN11* expression can confer PARP inhibitor resistance (Lok et al., 2017; Murai et al., 2016). Interestingly, this appears to make the cells specifically vulnerable to the combination of PARP and ATR

inhibitors (Murai et al., 2016), thereby providing alternative treatment options in case *SLFN11* cannot be induced by epigenetic modulation.

Altogether, the work of Gardner et al. (2017) suggests a therapeutic strategy in which standard chemotherapy combined with EZH2 inhibitors might prevent chemotherapy resistance of SCLC during first-line treatment while re-sensitizing tumors with acquired chemoresistance and loss of *SLFN11* expression as second-line therapy. Since the drugs are available, this can be readily tested in clinical trials. Furthermore, these studies open exciting new perspectives for treating other cancers that easily escape cytotoxic treatment such as ovarian cancer, for which it is encouraging that *SLFN11* inactivation has been observed as a predictor of poor platinum response (Nogales et al., 2016). Of note, although *EZH2* overexpression is mostly associated with oncogenesis, the observation that EZH2 loss of function can drive chemoresistance in AML (Göllner et al., 2017) or promote the specification of more aggressive NSCLC (Serresi et al., 2016) indicates that the effects of epigenetic modifying drugs are highly context dependent, and, therefore, their use urges for some caution.

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