IN THE SPOTLIGHT

A Genetic Snapshot of Small Cell Lung Cancer

Rafael Rosell[1,2] and Luciano Wannesson[3]

Summary: Overexpression of PARP1 and enhancer of zeste homolog 2 (EZH2) has been identified in an integrated analysis of multiple proteins involved in intracellular signaling pathways in small cell lung cancer (SCLC) cell lines. The knockdown of PARP1 and EZH2 abrogated SCLC growth. These findings provide the basis for novel predictive markers and new treatment strategies in SCLC, for which there is now a paucity of available therapies. *Cancer Discov; 2(9); 769–71. ©2012 AACR.*

Commentary on Byers et al., p. 798 (3).

Small cell lung cancer (SCLC) represents 13% of all newly diagnosed cases of cancer worldwide, or more than 180,000 cases per year. In contrast with non–small cell lung cancer (NSCLC), it is not associated with specific somatic mutations (1). The prognosis for patients with SCLC has not improved and treatment has remained substantially the same for the last 25 years. The first-line treatment of choice in extensive-stage SCLC is 4 to 6 cycles of etoposide combined with cisplatin or carboplatin, with a median survival of 8 to 13 months and a 2-year survival rate of 5%. For patients with limited-stage disease, the standard treatment is the same chemotherapy regimen with the addition of thoracic radiotherapy, and median survival is 15 to 20 months, with a 2-year survival rate of 20% to 40% (1). The *RB* and *TP53* tumor suppressor genes are both mutated in more than 90% of human SCLCs. Recent data indicate that *p130* mRNA levels are downregulated in SCLC. Similarly, loss of E2F4, a major partner for p130, partially suppresses lung neuroendocrine hyperplasias in *RB*-mutant mice. The loss of p130 accelerates the development of SCLC in *RB/TP53*-mutant mice (2).

In this issue of *Cancer Discovery*, Byers and colleagues (3) identify significant differences in signaling pathways between SCLC and NSCLC. Using reverse phase protein arrays, they studied the expression of 193 proteins in 34 SCLC and 74 NSCLC cell lines. PARP1 and enhancer of zeste homolog 2 (EZH2) were 2 of the most overexpressed proteins in SCLC. SCLC growth was abrogated by knockdown of PARP1 and EZH2. Intriguingly, SCLC cell lines were significantly more sensitive to PARP inhibitors than were NSCLC cell lines. PARP inhibition downregulated key components of the homologous recombination pathway, such as RAD51 and BRCA1. The authors observed lower levels of total and phospho-Rb and higher levels of E2F1 in SCLC than in NSCLC cell lines. They posit that because of the loss of RB, E2F1 expression leads to the activation of several E2F1 targets in SCLC cells, such as EZH2, thymidylate synthase (TS), and several components of DNA repair pathways, including 53BP1 (3). In contrast, NSCLC cells had higher expression of receptor tyrosine kinases that heterodimerize with EGFR, including HER2, MET, and AXL. The differential expression of PARP1 was confirmed by Western blotting and by mRNA analysis. The mRNA levels in SCLC were higher for *PARP1, EZH2, BCL2, PRKDC* (DNA PKcs), and proliferating cell nuclear antigen (*PCNA*). PARP1 expression was also examined in 318 cell lines derived from 30 types of primary cancer. Interestingly, SCLC cells showed the highest PARP1 expression. Immunohistochemistry confirmed that PARP1 was more highly expressed in SCLC than in NSCLC.

The most intriguing finding is that the majority of SCLC cells were sensitive to the use of PARP inhibitors, such as AZD2281, in contrast to the majority of NSCLC cell lines, which were resistant, including the A549 cell line. Because *BRCA1/2* mutations and PTEN loss are markers of PARP inhibition in breast and ovarian cancers, the authors compared PARP inhibition in the *BRCA1*-mutated HCC1395 breast cancer cell line and in the *PTEN*-mutated MDA-MB-468 breast cancer cell line (3). Both of these cell lines were sensitive to PARP inhibitors, but not more so than the SCLC cell lines. Finally, PARP inhibition led to the downregulation of *RAD51* and other essential DNA repair genes, such as *PCNA* and *BRCA1*, as well as other E2F1 targets, such as *TS* (3).

SCLC is one of the most hypoxic tumors (1), and hypoxia causes BRCA1 and RAD51 downregulation by stimulating E2F4/p130 occupancy of the *BRCA1* and *RAD51* promoters. In line with the findings of the authors (3), PARP inhibitors have been proven to cause *BRCA1* and *RAD51* downregulation at the transcriptional level via induction of E2F4/p130 binding to the *BRCA1* and *RAD51* promoters (4). PARP inhibition leads to the formation of double-strand DNA breaks that cannot be repaired in tumors that lack efficient homologous recombination. The fact that PARP inhibitors can also suppress the expression of BRCA1 and RAD51, 2 key components of homologous recombination, makes the findings of the Heymach group (3) particularly relevant. In fact, survival

Authors' Affiliations: [1]Catalan Institute of Oncology, Badalona; [2]Pangaea Biotech, USP Dexeus University Institute, Barcelona, Spain; and [3]Oncology Institute of Southern Switzerland, Locarno, Switzerland

Corresponding Author: Rafael Rosell, Medical Oncology Service, Catalan Institute of Oncology, Hospital Germans Trias i Pujol, Ctra Canyet, s/n, 08916 Badalona, Barcelona, Spain. Phone: 34 93 497 89 25; Fax: 34 93 497 89 50; E-mail: rrosell@iconcologia.net

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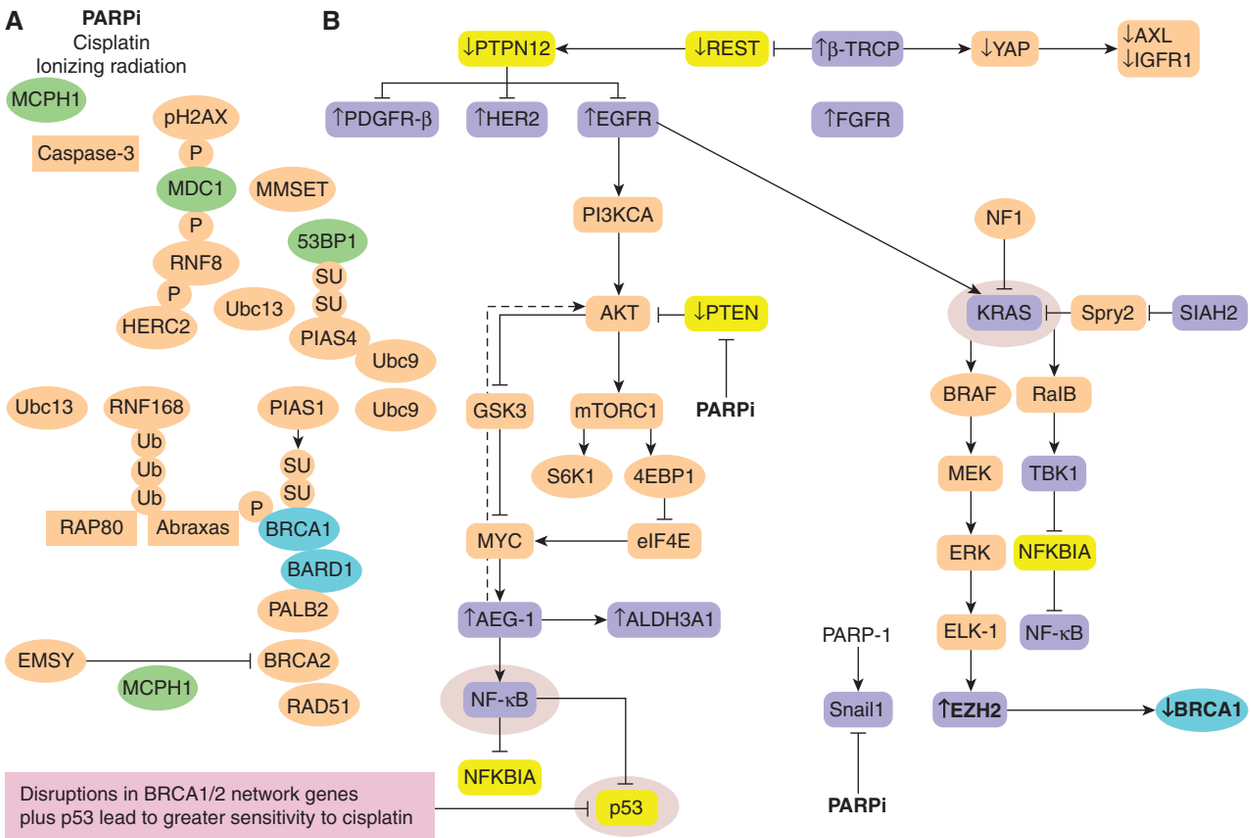

Figure 1. **A,** Signaling pathways depicting the position of BRCA1, RAD51, and 53BP1 in homologous recombination **B,** The role of EZH2 and its relationship with BRCA1 in breast cancer is also illustrated. Other signaling components in triple-negative breast cancers are also shown. Green, tumor suppressors upstream of the homologous recombination pathway; blue, tumor suppressors downstream of the homologous recombination pathway; purple, oncogenes; yellow, tumor suppressors. BC, breast cancer; P, phosphorylation; PARPi, inhibitor; SU, sumoylation; TNBC, triple-negative breast cancer; Ub, ubiquitination. For further explanation, see ref. 5. (Figure reproduced, with permission, from ref. 5.)

by colony formation of A549 NSCLC cells decreased under hypoxic conditions when treated with PARP inhibitors (4). As the authors suggest (3), clinical trials with PARP inhibitors are warranted in SCLC. However, when predicting the efficacy of PARP inhibitors, we should consider not only PARP1 expression but also the potential loss of p130, which could abrogate the effect of PARP inhibition on BRCA1 and RAD51.

Resistance to PARP inhibitors may be related to the loss of 53BP1, as occurs in triple-negative, BRCA1-mutated breast cancer. BRCA1 displaces 53BP1 from double-strand breaks, enabling resection at the break site by factors such as CtIP, which promotes RPA loading onto single-stranded regions of DNA. RPA is displaced by RAD51, leading to error-free template-directed repair of the double-strand break. However, in cells with loss of BRCA1, 53BP1 is not displaced, and DNA repair is abrogated (5). Because the authors found that 53BP1 was overexpressed in the SCLC lines (3), we can speculate that the high sensitivity to PARP inhibitors in the SCLC cell lines may be due in part to the effect of the 53BP1 expression.

In response to DNA double-strand breaks caused by cisplatin chemotherapy, MDC1 binds to γH2AX and controls the formation of damage-induced 53BP1 and BRCA1 foci, in part by promoting efficient H2AX phosphorylation. Other proteins, such as MCPH1, bind to BRCA2 and regulate the localization of BRCA2 and RAD51 at sites of DNA damage (Fig. 1). BRCA1 was identified as a differential modulator of chemotherapy response (5), and the decrease in the expression of BRCA1 and RAD51 induced by chronic hypoxia could offset chemoresistance and increase sensitivity to cisplatin, although not to paclitaxel (6). Therefore, several lines of evidence indicate that PARP inhibitors can be synergistic with cisplatin-plus-etoposide in SCLC.

In a subgroup of triple-negative breast cancers, RB and TP53 were inactivated, with some genetic traits that are similar to those identified in SCLC. In triple-negative breast cancers, overexpression of FGFR2 has been observed (5), as has also been described in SCLC (7), making FGFR2 a potential target for treatment.

Intriguingly, the results of the Heymach group are similar to those regarding the SV40 T/t antigen intrinsic 120-gene signature, in which 85 of the genes are closely related to p53, pRB, and E2F genetic networks and in which EZH2 was also upregulated (8). Heymach's group has identified the overexpression of EZH2 in SCLC, indicating that it could be an important target for treatment. EZH2 is a histone modifier protein that functions as a methyltransferase at lysine 27 of histone H3. EZH2 is also a member of the polycomb group of proteins and belongs to polycomb repressive complex 2.

Overexpression of EZH2 has been associated with poor outcome in prostate and breast cancers. EZH2 negatively regulates the expression of DAB2IP, which is a unique scaffolding protein that regulates several signaling pathways, including apoptosis signal-regulating kinase 1 (ASK1). ASK1 can activate several proapoptotic proteins, including BIM (9). High EZH2 protein levels have been associated with upregulated expression of AKT and decreased nuclear expression of phospho-BRCA1 in breast cancer (10). It has also been shown that overexpression of EZH2 downregulates *BRCA1* mRNA levels in estrogen-negative breast cancer (5). It could be of interest to examine the *EZH2:BRCA1* mRNA ratio in SCLC patients, although in the report by the Heymach group (3), it seems that BRCA1 and EZH2 could both be overexpressed. In our experience, the expression of both BRCA1 and EZH2 was significantly higher in SCLC than in NSCLC patient tumors (11). Therefore, the inverse relationship observed in breast cancer may not hold true for SCLC. Interestingly, the MDA-MB-468 breast cancer cell line, which was very sensitive to PARP inhibitors (3), also harbors *EGFR* amplification and has high EZH2 expression. This cell line is sensitive to mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitors. In breast cancer, the activation of the MEK/ERK1/2/ELK-1 pathway leads to EZH2 overexpression (ref. 12; Fig. 1). What is not known is if overexpression of EZH2 in SCLC also downregulates BRCA1 and if the elevated levels of EZH2 could be a consequence of the activation of the MEK/ERK1/2/ELK-1 pathway. In this case, the high expression of EZH2 could be druggable with MEK inhibitors.

The fundamental findings of the authors can lead to further insight into SCLC. For example, loss of miR-26a has been related to increased expression of EZH2 and AEG-1 (also known as MTDH), which confers chemoresistance (13). The identification of elevated expression of PARP1 in SCLC paves the way for introducing novel effective targeted therapies that can lead to definite progress in the treatment of this disease, in which trials with BCL2 inhibitors currently are being conducted (7).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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