MCAM Mediates Chemoresistance in Small-Cell Lung Cancer via the PI3K/AKT/SOX2 Signaling Pathway


Abstract

Despite favorable responses to initial therapy, small-cell lung cancer (SCLC) relapse occurs within a year and exhibits resistance to multiple drugs. Because of limited accessibility of patient tissues for research purposes, SCLC patient-derived xenografts (PDX) have provided the best opportunity to address this limitation. Here, we sought to identify novel mechanisms involved in SCLC chemoresistance. Through in-depth proteomic profiling, we identified MCAM as a markedly upregulated surface receptor in chemoresistant SCLC cell lines and in chemoresistant PDX compared with matched treatment-naïve tumors. MCAM depletion in chemoresistant cells reduced cell proliferation and reduced the IC50 inhibitory concentration of chemotherapeutic drugs in vitro. This MCAM-mediated sensitization to chemotherapy occurred via SOX2-dependent upregulation of mitochondrial 37S ribosomal protein 1/ATP-binding cassette subfamily C member 1 (MRP1/ABCC1) and the PI3/AKT pathway. Metabolomic profiling revealed that MCAM modulated lactate production in chemoresistant cells that exhibit a distinct metabolic phenotype characterized by low oxidative phosphorylation. Our results suggest that MCAM may serve as a novel therapeutic target to overcome chemoresistance in SCLC. Cancer Res; 77(16); 4414–25. ©2017 AACR.

Introduction

Small-cell lung cancer (SCLC) is an aggressive and highly metastatic lung cancer subtype, accounting for about 10% to 20% of lung cancer cases (1, 2). The 5-year survival rate has remained dismal at 7%, and systemic treatment options for patients with SCLC have remained unchanged (3). Standard first-line treatment of SCLC includes cisplatin or carboplatin in combination with etoposide, cyclophosphamide, vincristine, or doxorubicin, which leads to complete remission in a vast majority of patients (4). SCLC is highly responsive to chemotherapy at the start of treatment. However, relapse and resistance to treatment eventually contributes strongly to poor prognosis.

Established mechanisms of chemoresistance in cancer include cellular pathways associated with DNA damage and repair, apoptosis, NOTCH signaling, and FGFR signaling (5, 6). Agents targeting these pathways that have shown promise for other tumor types have been investigated in SCLC without demonstrable clinical benefit (7). Consequently, there is a need to elucidate novel molecular mechanisms involved in chemoresistance in SCLC. Limited translational success is largely attributed to the lack of sufficient tumor materials from SCLC patients. In addition, data on SCLC in public databases, such as TCGA or oncomine, are sparse. SCLC patient-derived xenografts (PDX) have provided the best opportunity to address the above-mentioned limitations, as PDXs replicate the biology and clinical properties of the original patients’ tumors as compared with other animal models (8).

A comprehensive map of the actual proteome in SCLC, in particular, chemoresistant phenotype is still needed. Herein, for the first time, we have investigated mechanisms of SCLC chemoresistance with a focus on the cell surface proteins. We initially compared the proteomic and metabolomic profiles of SCLC cell lines to identify molecular features associated with chemoresistance. MCAM was identified as a markedly overexpressed protein in SCLC chemoresistant as well as patient tumors. MCAM is a cell adhesion molecule initially identified as melanoma-specific cell adhesion molecule (9). MCAM is also involved in several cellular processes, including cell invasion, migration, angiogenesis, epithelial–mesenchymal transition (EMT), immune response, and signal transduction (10). In addition, MCAM has low expression levels in normal tissue, primarily restricted at intracellular junctions of endothelial cells (10, 11). Previous studies have shown differential expression of MCAM in primary tumors correlated with metastasis and poor prognosis in several cancers, showing its significant potential in cancer therapy (12–14). Despite the identification of MCAM expression in the lung adenocarcinoma (13), its expression and role in SCLC has not been reported yet. We
further investigated the effect of MCAM through knockdown experiments and the consequences of its overexpression on SCLC chemoresistance and cellular functions.

**Materials and Methods**

**Cell lines and treatment**

The human SCLC cell lines H69, H82, DMS79, H209 and H136, H69AR were obtained from the ATCC in 2011 and 2014, respectively. All cells grew in RPMI1640 with 10% FBS and a 1% penicillin/streptomycin cocktail. For stable isotope labeling with amino acids in cell culture, cells were allowed to grow for seven passages in RPMI1640 supplemented with $^{13}$C-lysine and $^{2}H_{19}6$, H69AR were obtained from the ATCC in 2011 and...

**Proliferation and colony formation assays**

For proliferation assays, $8 \times 10^5$ SCLC cells were seeded in triplicate and assayed using MTS reagent (CellTiter 96 Aqueous One Solution Cell Proliferation Assay; Promega). For colony formation assays, 200 cells were seeded in 6-well plates in triplicate and allowed to grow for 20 days. Cells were fixed in 6% glutaraldehyde with 0.5% Crystal violet and visualized under the Nikon microscope. Colony areas were measured using the ImageJ software program.

**In vitro drug sensitivity assay**

SCLC cells were seeded in 96-well plates at a density of $1 \times 10^4$ cells per well and treated in medium with doxorubicin, cisplatin, or etoposide for 24 hours. Cell survival was analyzed using a CellTiter Glo assay (Promega) according to the manufacturer’s instructions. The range of drug concentrations was chosen to obtain IC$_{50}$ values for SCLC cell lines. After incubation with 100 $\mu$L of CellTiter Glo reagent for 10 minutes, the luminescence was measured. Luminescence reading from the cells incubated without the drugs was used for 100% survival and to calculate the IC$_{50}$ of each drug. The data for CellTiter Glo assay was collected from five technical and three biological replicates for each sample.

**Flow cytometric analysis**

SxSCLC cells were treated with doxorubicin, cisplatin, or etoposide for 24 hours and then collected for apoptosis and cell-cycle analyses. For Annexin V analysis, cells were incubated with Annexin V-FITC and propidium iodide (PI) for 15 minutes at room temperature in the dark. Samples stained with Annexin V-FITC and PI were diluted in 400 $\mu$L of Annexin V-binding buffer and immediately examined using a FACS machine. Stained cells were immediately subjected to flow cytometric analyses using a Gallios flow cytometer (Beckman Coulter). Early apoptotic cells were defined as cells with Annexin V–positive and PI-negative staining. Late apoptotic and nonviable cells were defined as having both Annexin V–positive and PI-positive staining.

**IHC**

The paraffin-embedded PDX tissues sections (5 $\mu$m) on glass slides were deparaffinized and hydrated, and antigen retrieval was performed using a decloaker with a target retrieval solution (pH, 6.0; Dako). The intrinsic peroxidase activity was blocked using 3% methanol and hydrogen peroxide for 10 minutes, and a serum-free protein block (Dako) was used for 5 minutes to block nonspecific antibody binding. The slides were then incubated with antibodies against human MCAM (ab5769, 1:200 dilution; Abcam), EGFR (ab52893, 1:100, Abcam), EphA2 (#6997, 1:200, Cell Signaling Technology), ITGB1 (ab52971, 1:250, Abcam), and JAG1 (ab109536, 1:100, Abcam) overnight at 4°C. After being washed three times in Tris-buffered saline, the slides were then incubated for 30 minutes with Dako EnVision+ Dual Link at room temperature. Slides were incubated with Dako chromogen substrate for 5 minutes and counterstained with hematoxylin. Formalin-fixed, paraffin-embedded, whole-section specimens...
Figure 1. MCAM in SCLC chemoresistant cells. A, Schematic diagram of the proteomics workflow. B, Heatmap cluster of SCLC cell line surface proteins in H69AR compared with other SCLC cell lines. C, Immunoblot of differentially expressed proteins on cell surface of H69AR compared with other SCLC cell lines. D, Bar chart of mass spectrometry (MS) counts for five most abundant cell surface receptors on H69AR compared with other SCLC cell lines. E, Schematic diagram of chemoresistant tumor formation in SCLC PDX models. Highly abundant expression of MCAM in chemoresistant PDX tissues compared with their respective naive PDX tissues. Representative images of MCAM IHC at ×20 (scale bar, 100 μm) and ×40 (scale bar, 50 μm) magnification.
with the primary antibodies omitted were used as negative controls.

Mathematical modeling for regulation of MCAM
We developed a mathematical model that incorporates the regulations among MCAM, SOX2, PI3K, and CREB1 and their association with chemoresistance.

Oxygen consumption rate measurement
Oligomycin, an inhibitor of ATP synthase, was prepared from 1,000× stock at a concentration of 10 mmol/L in DMSO. FCCP, an ionophore and strong mitochondrial depolarizer, was prepared from 1,000× stock solutions at concentrations of 10 mmol/L in DMSO. Rotenone, a potent inhibitor of mitochondrial complex I, and antimycin A, a strong suppressor of mitochondrial complex III, were solubilized from 1,000× stock at a concentration of 5 mmol/L in DMSO. To measure SCLC–cell oxygen consumption rates (OCR), 6×10⁴ cells from each cell line were seeded into each well of an XF96 microplate 16 hours before the experiment. Immediately before the OCR measurement, culture medium of the cells was replaced by an assay medium (low-pyruvate, and 1 mmol/L L-glutamine) and incubated for 1 hour at 37°C, 5% CO2, to reach the final working 1× concentrations. After 5 minutes of incubation to expose cancer cells equally to chemical inhibitors, the OCR was measured again. Data were analyzed using the Prism software program (GraphPad Software) with the statistical significance of the results being assessed using the paired t-test. P < 0.05 was considered statistically significant. Data were analyzed using the Prism software program (GraphPad Software) unless otherwise stated. All experiments were independently repeated at least three times.

Statistical analysis
All bar and line graphs represent means and SDs. The error bars in OCR line graphs are SDs. The unpaired t-test was used to compare differences between two groups. For comparison of more than two groups, one-way ANOVA was used. A P value less than 0.05 was considered statistically significant.

Results
Increased MCAM expression in chemoresistant SCLC
In-depth proteomic multicompartment profiling was performed to quantify proteomic changes associated with chemoresistance of SCLC cells (Fig. 1A). We used H69 and H69AR as a paired SCLC-chemosensitive and chemoresistant cell line. Metastasis and chemoresistance in cancer are linked phenomena (17); hence, we used H82 representing a metastatic SCLC cell line. We also used DMS79, a SCLC cell line established from the tumor cells of a patient who had undergone chemotherapeutic and radiation treatment, as assumed to be a surviving fraction of SCLC cells. We used the Ingenuity Pathway Analysis (IPA) (Qiagen) and STRING databases to identify pathways associated with MCAM expression and to perform network analyses. Proteins were selected on the basis of IPA and STRING results. From this list, we focused on cell surface receptors that were further assessed by immunoblotting (Fig. 1C). To narrow our list of candidate targets, we focused on cell surface receptors that exhibited the most robust differences between chemoresistant SCLC cells compared with other cell lines. Five surface membrane
receptors (EGFR, JAG1, ITGB1, EPHA2, and MCAM) were found most highly expressed in chemoresistant SCLC cells compared with the other cell lines (Fig. 1D). We validated our results by immunoblotting in these SCLC cell lines also including H196, which has similar chemoresistant properties as of H69AR, and H209, which is chemosensitive to the chemotherapeutic drugs. Increased expression of H69AR surface enriched protein on DMS79 and H196 is in concordance to their higher IC50 values for chemotherapeutic drugs compared with H69, H82, and H209 (Fig. 1C; Supplementary Fig. S1B and S1C). We found that DMS79 and H196 closely related to H69AR, whereas H82 and H209 resemble H69 in terms of their surface protein expression. Next, we examined established PDXs derived from SCLC patients for expression of these receptors. The PDX models replicate the biology and clinical properties of the original patients’ tumors (8). We observed increased MCAM expression in chemoresistant tumors compared with matched treatment-naive tumors (Fig. 1E; Supplementary Fig. S1D). We also observed increased MCAM expression as early as after two cycles of carboplatin (Supplementary Fig. S1E) in contrast with EGFR, JAG1, or ITGB1, which did not exhibit any difference in immunostaining between chemosensitive and chemoresistant tumors. EPHA2 was undetectable by immunostaining in any of the PDXs (Supplementary Fig. S1F).

MCAM expression is associated with a mesenchymal phenotype, increased proliferation, and colony formation

MCAM expression was highly correlated with a mesenchymal transition-related gene expression pattern in SCLC cell lines annotated in the Cancer Cell Line Encyclopedia database (Supplementary Fig. S2A). Concordantly, we also observed altered expression of multiple EMT markers, including reduced expression of CDH1 and NCAM and increased expression of VIM, CDH2, LGALS1, and COL1A1 (Fig. 2A) in H69AR cells. Interestingly, LGALS1 has also been reported as a ligand for MCAM in melanoma and endothelial cells (18, 19). Ingenuity Pathway Analysis revealed TGFβ and ZEB1 as key upstream regulators of altered gene expression in chemoresistant cells (Supplementary Fig. S2B–S2D). Higher expression of the EMT regulators ZEB1 and TWIST1 in H69AR cells compared with H69 cells was confirmed by immunoblotting (Fig. 2B).

Knockdown of MCAM expression in chemoresistant cells using RNAi had no effect on mesenchymal-related protein expression (Fig. 2C–E; Supplementary Fig. S3A–S3C). However, MCAM knockdown in chemoresistant cells significantly decreased cell proliferation (P < 0.001, ANOVA; Fig. 2F; Supplementary Fig. S3D and S3E) and colony-forming rates (P < 0.01, unpaired t test; Fig. 2G).

**Figure 2.** Effect of MCAM knockdown on SCLC chemoresistant cells with mesenchymal phenotype. A, Bar chart for mass spectrometry (MS) counts of mesenchymal-related proteins in SCLC cell lines. B, Immunoblot for epithelial-to-mesenchymal transition-related proteins and transcription factors. C and D, Knockdown efficiency of shRNAs for MCAM at transcriptional (C) and translational (D) levels. Data were pooled from three biological replicates (n = 3). E, Highly efficient reduction of MCAM protein on cell surface after MCAM knockdown was observed using flow cytometry. F, Stable knockdown of MCAM statistically significantly (P < 0.01, ANOVA) reduced the cell proliferation in chemoresistant H69AR cells. Data were pooled from three biological replicates (n = 3). G, H69AR cells statistically significantly (P < 0.01, unpaired t test) lost their colony-forming abilities after stable knockdown of MCAM. Colonies were stained with Crystal violet. Error bars represent means ± SD from three biological replicates (n = 3). **, P < 0.05; ***, P < 0.01; ****, P < 0.001 vs. control shRNA group.
MCAM expression is a determinant of SCLC chemoresistance and sensitivity.

To determine the effects of MCAM expression on chemoresistance of SCLC cells, we treated shMCAM-H69AR cells with varying doses of chemotherapeutic drugs (doxorubicin, cisplatin, and etoposide). MCAM knockdown in chemoresistant cells increased chemosensitivity, with significant reductions ($P < 0.001$, ANOVA) in the IC$_{50}$ and cell survival (Fig. 3A–D; Supplementary Fig. S3F and S3G). Reduced MCAM expression led to a marked increase in apoptosis after chemotherapy as determined by flow cytometry (Fig. 3E). Immunoblotting was performed for proteins in the apoptotic pathway. Of note, expression of p-BAD, a proapoptotic

Figure 3.
Effect of MCAM knockdown on SCLC chemoresistance. A–C, The cell proliferation rate was measured using CellTiter Glo assay. Data were pooled from three biological replicates ($n = 3$). Values are presented as percentage of cell proliferation in doxorubicin (A), cisplatin (B), and etoposide (C)-treated and untreated cells. **, $P < 0.001$ (one-way ANOVA; compared with corresponding negative control groups). D, Bar graph representing effect of MCAM modulation on drug sensitivity of SCLC cell lines. Data were pooled from three biological replicates ($n = 3$). E, MCAM knockdown increased cell apoptosis in SCLC cells after chemotherapy ($n = 3$). Representative FACS profiles are shown, on which cell population in the quadrant of Annexin V+/DAPI represents apoptotic cells. F, Chemoresistant H69AR cells (parental and MCAM knockdown clones) were treated with doxorubicin (170 μmol/L) for 24 hours, and cell lysates were immunoblotted for MCAM, p-BAD, and cleaved caspase-3.
protein, increased after MCAM knockdown. In addition, cleaved caspase-3 markedly increased during chemotherapeutic treatment confirming an apoptotic mechanism (Fig. 3F). We also observed increased cell-cycle arrest at G0–G1 and G2–M after treatment with doxorubicin or cisplatin and etoposide, respectively, in shMCAM-H69AR compared with H69AR (Supplementary Fig. S4A–S4C).

To determine whether MCAM expression is sufficient to induce SCLC chemoresistance, we ectopically expressed MCAM in the chemosensitive H69 cell line. Overexpression of MCAM resulted in a slight increase in the cell proliferation; however, this was not statistically significant (Fig. 4A–C; Supplementary Fig. S5A). MCAM overexpression resulted in markedly increased chemotherapeutic IC50 values and cell survival (Figs. 3D and 4D). These findings were corroborated by reductions in apoptosis and reduced cell-cycle arrest at G0–G1 and G2–M following treatment with doxorubicin or cisplatin and etoposide, respectively (Fig. 4E; Supplementary Fig. S5B).

Regulation of MCAM via PI3K/AKT pathway in a SOX2/CREB1–dependent manner

xPI3K/AKT signaling emerged as an upregulated pathway in chemoresistant SCLC cells (Table 1). We tested whether MCAM regulates PI3K/AKT activation. MCAM knockdown considerably reduced the activation state of PI3K/AKT pathway based on phosphoprotein analysis (Fig. 5A; Supplementary Fig. S4D). Moreover, inhibition of PI3K activity using LYS29004 markedly decreased MCAM expression, suggesting a potential bidirectional regulation (Fig. 5B). MCAM expression is known to be regulated via CREB1 (20), and CREB1 activation is dependent on the AKT pathway (21). The gene for the transcription factor SOX2 is frequently amplified in SCLC (22), and its expression is also regulated via AKT (23). Hence, we examined the effect of SOX2 knockdown and PI3K inhibition on CREB1 and MCAM protein expression levels in chemoresistant SCLC cells. Both SOX2 inhibition and LYS29004 treatment reduced CREB1 and MCAM protein expression (Fig. 5C and D). However, CREB1 knockdown reduced MCAM but did not affect SOX2 expression (Fig. 5E).

Our findings led to a mathematical model for a regulatory network mediating chemoresistance of SCLC that can behave as a bistable switch [i.e., cells can attain one of two stable-steady phenotypes, chemosensitive or chemoresistant, and can transit from one to the other based on the expression levels of SOX2 and MCAM (Fig. 5F and G; Supplementary Data)].

Figure 4. Effect of MCAM overexpression on SCLC chemoresistance. A and B, Efficient overexpression of MCAM in H69 cells both at transcriptional (A) and translational (B) level. Data were pooled from three biological replicates (n = 3). Error bars, means ± SD; **, P < 0.001. C, Increased MCAM expression on cell surface after MCAM overexpression was confirmed by flow cytometry. D, The cell proliferation rate was measured using CellTiter Glo assay. Values are presented as percentage of cell proliferation in doxorubicin-, cisplatin-, etoposide-treated, and untreated cells (n = 3). E, MCAM overexpression markedly decreased cell apoptosis in SCLC cells after chemotherapy. Representative FACS profiles are shown, on which cell population in the quadrant of Annexin V+/DAPI represents apoptotic cells.
Given our observation of a bidirectional regulation of MCAM and PI3K/AKT pathway activation, we further assessed whether MCAM regulates known PI3K/AKT–regulated targets that have been associated with chemoresistance. Of particular interest is the ABCC transporter, MRP1, which plays a key role in the chemoresistance of several cancers (24, 25) and is regulated by PI3K/AKT (26, 27). We observed markedly decreased expression of MRP1 protein following MCAM knockdown, suggesting that MRP1 regulation is associated with MCAM expression in chemoresistant cells (Fig. 5H). We also observed reduced levels of NRF2, a known modulator of MRP1 in SCLC (28), in MCAM knockdown cells (Supplementary Fig. S4D).

Increased lactate and low oxidative phosphorylation are key features of SCLC chemoresistance

PI3K/AKT activation promotes increased glycolysis rather than oxidative phosphorylation in cancer (29). MRPI is known to transport glutathione and glutathione conjugates out of the cell. Thus, the association between MCAM, PI3K/AKT activation and MRPI inherently suggests metabolic rewiring, a characteristic that may in part be regulated by MCAM.

To determine whether H69AR exhibited a unique metabolic profile compared with the parental H69 cell line, we conducted Ingenuity Pathway Analysis, focusing on proteins that exhibited at least 2-fold changes in TCE and at least five MS/MS counts in both cell lines (Supplementary Table S2). We found that mitochondrial dysfunction was the most altered pathway (Fig. 6A). Moreover, many of the top perturbed pathways linked to altered metabolism included oxidative phosphorylation, gluconeogenesis, glycolysis, pentose phosphate pathway, and mTOR signaling (Fig. 6A). We thus evaluated the basal OCRs in the chemoresistant H69AR and other SCLC cell lines (H69, H82, and DMS79) using a Seahorse assay (Fig. 6B). The basal OCR was significantly lower ($P < 0.001$, unpaired $t$ test) in the chemoresistant cell line compared with the other SCLC cell lines, demonstrating that the basal metabolic conditions in drug-resistant SCLC cell lines favor elevated aerobic glycolysis and reduced oxidative phosphorylation. Lactate production rate was significantly higher ($P < 0.001$, unpaired $t$ test) in H69AR cells than in H69 cells (Fig. 6C). These findings are consistent with a shift toward a glycolytic phenotype associated with PI3K/AKT activation (29). Lactate production decreased significantly ($P < 0.01$, unpaired $t$ test) after MCAM knockdown (Fig. 6D), suggesting a potential shift away from glycolysis. We also observed reduced levels of glutathione reductase (GSR), a downstream effector molecule of NRF2 in MCAM knockdown cells (Supplementary Fig. S4D).

Discussion

The rapid emergence of chemoresistance in SCLC following treatment is a key contributor to poor survival. Therefore, there is critical clinical need to develop novel strategies that overcome chemoresistance and provide significant survival benefits to patients.
Analysis of in-depth proteomic profiling for TCE and cell surface enriched proteins of SCLC cell lines revealed that the chemoresistant cells have marked distinct surface and TCE profiles. In particular, proteins related to integrin signaling and PI3/AKT signaling, such as ITGB1, ITGB5, ITGA2, ITGA4, VCL, ZYX and CTNNB1, PP2A, GYS, respectively, were observed to be upregulated in TCE as well as enriched on surface of chemoresistant cells. The integrin–ECM interactions are a well-known phenomenon for cell survival and drug resistance in various cancers, including solid and hematologic malignancies (30). On the other hand, Akt signaling can induce transformation and renders tumor cell resistant to chemotherapeutic agent through its antiapoptotic activity and induction of cell-cycle progression (31–33). We also observed differential expression of proteins related to cancer stem cell (CD44, ALDH3A2, ALDH7A1, EpCAM), EMT (CDH1, Vim, CDH2), and receptor signaling (EGFR, LGALS1, EPHA2, JAG1) that can modulate chemoresistance in cancer cells (34–39).

Our enriched cell surface data identified MCAM among the highly differentially upregulated surface proteins in chemoresistant compared with other SCLC cells. MCAM (also designated as CD146 or MUC18) is a marker of endothelial cell lineage (10). One of the limitations of our study is lack of chemoresistant tissues from SCLC patients. However, SCLC PDX models can replicate the biology of cancer in patients and hence are superior to traditional xenograft tumor models. We thus incorporated PDX models as a component for validation of our proteomics data. We observed markedly increased expression of MCAM protein in chemoresistant than in chemonaive SCLC PDXs. Increased expression of MCAM on DMS79 but not on H82 and on PDX tissues even after two cycles of chemotherapeutic treatment suggested that the overexpression might be an early event for an acquired chemoresistance in SCLC cells. MCAM overexpression has been observed in several tumor types, including melanoma, prostate, pancreatic, lung, gastric, breast, and ovarian cancers (10). Altered expression of MCAM was associated with cell viability and colony-forming abilities.
in SCLC cells, which is in concordance with the reports of MCAM linked to altered cell proliferation, angiogenesis, metastasis, cell motility, and invasion (10, 40, 41). The modulation of chemoresistance through altered MCAM expression is a novel finding, which may be mediated by regulating the apoptosis-related protein expression. We also observed increased LGALS1 protein levels, a known ligand of MCAM (15, 16), in chemoresistant cells. MCAM and its ligand LGALS1 has been reported to regulate apoptosis in cancer cells (18, 19, 42–44), which further supports our findings.

Given that SCLC is a neuroendocrine tumor type, it is noteworthy that multidrug resistance in SCLC was associated with an EMT phenotype, suggesting that mesenchymal transition is a potentially important mechanism of survival for chemoresistant cells in SCLC, and analysis of CCLE (45) data revealed a strong association between MCAM and EMT. Zeng and colleagues (14) demonstrated that high MCAM expression in triple-negative breast cancers induced EMT and cancer stem cell properties. However, we observed that MCAM modulation has no significant effect on EMT properties of SCLC cells.

In melanoma, MCAM upregulation is dependent on PI3K/AKT pathway, which is a known contributor of chemoresistance in numerous malignancies (46). However, our results implicate that in chemoresistant SCLC cells, the relationship between MCAM and PI3K/AKT activation is bidirectional and postulated to be mediated via SOX2/CREB1 axis. These findings are also in concordance with those of recent studies, suggesting that SOX2 expression levels in malignant cells are dependent on AKT regulation (47–49). However, further studies needed to confirm this potential relationship.

To define the mechanism(s) by which MCAM can modulate chemoresistance, we evaluated known targets regulated by the PI3K/AKT pathway. Of relevance was MRP1, which has been previously implicated in promoting chemoresistance in numerous cancer types, including SCLC (26, 50). Knockdown of MCAM reduced MRP1 expression. MRP1 has a high affinity for exporting glutathione-conjugated metabolites and drugs (51). Multidrug-resistant H69AR cells exhibited greater Nrf2 activation than H69 cells, and its expression is associated with MRP1 regulation (28). Nrf2 regulates a number of metabolic pathways, including the induction of antioxidant pathways including the glutathione pathway, as well as NADPH production through G6PD, the rate-limiting enzyme in the pentose phosphate pathway (52). Thus, the observed reduction in MRP1, NRF2, and its downstream effector glutathione reductase due to MCAM knockdown suggests that MCAM works in concert with altered Nrf2 activation, a notion that is supported by the observation that activation of the PI3K/AKT pathway promotes Nrf2 activation in other cancer types (53).

In the current study, comparison of the proteome of chemoresistant to other SCLC cell lines revealed perturbations in various metabolic pathways, highlighted by dysregulation of mitochondrial function, gluconeogenesis, glycolysis, and the pentose phosphate pathway. Consistently, chemoresistant cells exhibited reduced basal oxygen consumption, elevated lactate production consistent with increased aerobic glycolysis, and reduced oxidative phosphorylation. Collectively, these findings implicate a metabolic rewiring of glucose metabolism and redox status, both of which inherently linked to PI3K/AKT and Nrf2 activation (29, 46, 53). Notably, lactate production reduced upon MCAM knockdown, implicating a reduction in glucose catabolism through the glycolytic pathway. Further studies, including stable isotope tracer studies, will be required to elucidate the role of MCAM in modulating cancer cell metabolism; however, these remain outside of the immediate scope of this study.

Our findings point to an important role for MCAM in SCLC chemoresistance. Moreover, targeting surface MCAM may serve as a novel therapeutic strategy to combat chemoresistance by modulating the activity and expression of PI3K, Nrf2, and MRP1, well-documented and interconnected contributors of drug resistance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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