Molecular Cancer Therapeutics

Sensitivity of Small Cell Lung Cancer to BET Inhibition Is Mediated by Regulation of *ASCL1* Gene Expression

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Abstract

The BET (bromodomain and extra-terminal) proteins bind acetylated histones and recruit protein complexes to promote transcription elongation. In hematologic cancers, BET proteins have been shown to regulate expression of *MYC* and other genes that are important to disease pathology. Pharmacologic inhibition of BET protein binding has been shown to inhibit tumor growth in *MYC*-dependent cancers, such as multiple myeloma. In this study, we demonstrate that small cell lung cancer (SCLC) cells are exquisitely sensitive to growth inhibition by the BET inhibitor JQ1. JQ1 treatment has no impact on MYC protein expression, but results in downregulation of the lineage-specific transcription factor *ASCL1*. SCLC cells that are sensitive to JQ1 are also sensitive to ASCL1 depletion by RNAi. Chromatin immunoprecipitation studies confirmed the binding of the BET protein BRD4 to the *ASCL1* enhancer, and the ability of JQ1 to disrupt the interaction. The importance of *ASCL1* as a potential driver oncogene in SCLC is further underscored by the observation that *ASCL1* is overexpressed in >50% of SCLC specimens, an extent greater than that observed for other putative oncogenes (*MYC*, *MYCN*, and *SOX2*) previously implicated in SCLC. Our studies have provided a mechanistic basis for the sensitivity of SCLC to BET inhibition and a rationale for the clinical development of BET inhibitors in this disease with high unmet medical need. *Mol Cancer Ther; 14(10); 2167–74. ©2015 AACR.*

Introduction

Bromodomains are protein interaction modules that recognize acetylation motifs commonly found on the unstructured tails of histones (1). A subclass of bromodomain proteins, known as the BET (bromodomain and extra-terminal) family, is composed of BRD2, BRD3, BRD4, and the testis-specific BRDT (2). The binding of BET proteins to acetylated histones allows the recruitment of chromatin regulators, such as transcription factors and nucleosome remodeling complexes, to specific chromatin sites resulting in targeted gene expression. There has been increasing interest in the BET family proteins fueled by the discovery of selective and potent small-molecule inhibitors that compete with acetylated histones in binding to BET family bromodomains. Studies using these inhibitors have revealed a role for BET proteins in controlling the expression of genes involved in an array of critical cellular processes. Deregulation of BET-mediated gene transcription has been proposed to underlie the etiology of a number of human diseases, including obesity, cardiovascular disorders, autoimmunity, and cancer (3).

Studies conducted with potent and selective small-molecule BET inhibitors demonstrated an important role of BET protein in NUT-midline carcinoma, multiple myeloma, and acute myelogenous leukemia (AML; refs. 4–9). The inhibitor JQ1 inhibits the growth of multiple myeloma and AML tumors by downregulating the expression of *MYC* (6–9). The identification of superenhancers, or large clusters of transcriptional enhancers, that regulate expression of oncogenes, such as *MYC*, has provided an explanation for how JQ1, and other inhibitors of chromatin regulators, may serve as an effective cancer therapy (10, 11). To date, JQ1 and other BET inhibitors have been found to inhibit the proliferation of cancer cell lines representing an array of tumor histologies (7, 12–15). From these studies, it is clear that BET inhibitors have therapeutic utility well beyond hematologic diseases. A consistent finding in these studies is that BET-sensitive tumor growth is not solely driven by *MYC* regulation, and that the mechanism of inhibition is likely to be dependent on cell context.

Here, we report on the observation that small cell lung cancers (SCLC) are exquisitely sensitive to JQ1. Through gene-expression profiling analyses, we identified achaete-scute homolog-1 (*ASCL1*) to be a BET target gene specifically expressed in BET-sensitive SCLC. ASCL1 is a basic helix-loop-helix transcription factor important to early development of neuroendocrine progenitor cells and in cancer cells with neuroendocrine features (16–18). Here, we show that JQ1 inhibits the growth of SCLC cells by downregulating *ASCL1* gene expression. This observation parallels the growth effects observed with JQ1 in multiple myeloma where in place of

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MYC the downregulation of *ASCL1* expression underlies JQ1 sensitivity in SCLC.

Materials and Methods

Cell lines, proliferation assay, and antibodies

The human multiple myeloma cell line KMS11 was obtained in 2013 from the Japanese Collection of Research Bioresources Cell Bank. The CAL-12T lung tumor cell line was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen in 2012. All other human lung tumor cell lines were obtained from the ATCC between 2012 and 2013. All cell lines were authenticated by short tandem repeat DNA profiling at the respective cell banks and were maintained as recommended by the suppliers. All cell lines were used up to no more than 25 continuous passages. Cell lines were plated in 96-well plates and JQ1 was added 24 hours later. Cell growth was determined 72 hours after compound addition, using the MTS dye conversion method (Promega). Antibodies for Western blot detection of MYC (#5605), SOX2 (#3579), and actin (#4967) were obtained from Cell Signaling Technology. Antibody to ASCL1 (#556604) was from BD Pharmingen. JQ1 was synthesized by Bristol-Myers Squibb Discovery Chemistry.

Caspase cleavage induction assay

Tumor cells were plated in a 384-well plate from BD Biosciences and cultured for 24 hours before treatment. Cells were loaded with CellPlayer caspase-3/7 reagent to a final concentration of 5 μmol/L in cell culture media containing JQ1. Cells were then incubated in an IncuCyte FLR Imaging System for 72 hours. Both fluorescence and phase-contrast images were acquired every 2 hours. Data analysis was performed using an object counting software (Essen Biosciences).

Expression profiling and RT-PCR analyses

Experimental design, cell growth, and treatment for expression profiling analysis were as previously described (19). Four lung cancer cell lines (DMS53, H2227, H1048, and H1694) were plated in 96-well plates, and JQ1 in 3-fold serial dilution covering six logarithm ranges in drug concentration was added 24 hours later. For global transcriptome analysis, total RNA was isolated at 2 hours of treatment using the RNeasy Mini Kit (Qiagen). RNA integrity was confirmed on a Bioanalyzer 2100 (Agilent Technologies) before labeling using 3′-IVT Express (Affymetrix). Microarray analysis was performed on the Human Genome U219 Array Plate using the Gene Titan MC instrument (Affymetrix). The resulting cell files were processed using the robust multi-array analysis algorithm (20). Probe sets on the HG-U219 array were mapped to loci using the criterion of >80% overall sequence identity to the loci annotated in NCBI's RefSeq database, release 39. The microarray data have been deposited into the ArrayExpress database under the accession number E-MTAB-3449 (http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-3449). The Sigmoidal Dose Response Search (SDRS) algorithm was applied as previously described (19), with the modification that a 1.1 multiple and no step function was used to set the 128 log-evenly distributed test values for C. For data visualization, the 12 intensity values for each treatment were scaled from 0 to 1, providing a view of the direction and consistency of change for each probe set. Note that where a consistent dose response is not present, this scaling result in a random pattern of high and low values across the dose series. The scaling may also result in a dose response being apparent, although fold change and/or *P* value criteria were not met. All heatmaps use the hierarchical clustering function of Partek Genomic suite v6.6, performed with euclidean distance metrics applied to the scaled data. *ASCL1* and *SOX2* mRNA abundance was measured by quantitative RT-PCR. The primers used were obtained from Applied Biosystems (#4351372 for *ASCL1* and #4331182 for *SOX2*).

siRNA knock-down

DMS53 cells were seeded at 1.7×10^5 cells per well in 6-well plates, and were transfected the following day using DharmaFECT 1 (Dharmacon). Four individual, ON-TARGETplus siRNAs for *ASCL1* and their SMARTPOOL mix were used at 25 nmol/L and cells were harvested at 72 hours posttransfection for ASCL1 protein level by Western blot analysis. A control siRNA with a scrambled sequence was also included to monitor potential off-target effects. Effect of the siRNA on cell viability was measured using CellTiterGlo Luminescence (Promega), and an siRNA to the kinesin KIF11 was included as a positive control (21).

Chromatin immunoprecipitation

H2227 and DMS53 cells were treated with DMSO or JQ1 (500 nmol/L) for 4 hours before they were fixed and processed for chromatin isolation. Chromatin immunoprecipitation was performed using an antibody to BRD4 (Bethyl Laboratories; A301-985A), or RNA polymerase II (Abcam; ab5095). A primer set (Active Motif human negative control set; cat. #71001) that amplifies a region in a gene desert on chromosome 12 was used as a background control. Mock control samples (input DNA) were amplified and sequenced to estimate noise and PCR bias. The 50-nt sequence reads obtained using Illumina HiSeq were mapped to the genome (hg19) using the BWA algorithm with default settings. Only reads that passed Illumina's purity filter, aligned with no more than 2 mismatches, and mapped uniquely to the genome were used in subsequent analyses. Peaks were detected using Partek Genomics Suite. Peak detection parameters were set with window size at 200 and average fragment size at 150. FDR cutoff for peak detection was set at <0.1%. Differential analysis of the detected peaks against mock control was done using *t* test statistics, and significance level was defined as *P* value less than 0.01. BRD4 binding to the ASCL1 enhancer was mapped using histone H3K27 acetylation marks previously documented by the ENCODE project (22).

Results

SCLC cell lines are sensitive to BET inhibition

JQ1 was previously found to inhibit the proliferation of lung tumor cell lines, although both of the previous studies were limited in scope as testing was done with only 24 or fewer cell lines (12, 14). JQ1 was tested on a total of 83 lung tumor cell lines in cell proliferation assays, and the IC_{50} values observed ranges from 9 nmol/L to greater than 10 μmol/L (Fig. 1A). It is apparent that the most sensitive cell lines are enriched in those of SCLC histology—15 of 18 SCLC lines tested have $IC_{50} < 1$ μmol/L, and 8 of the 10 most sensitive lung cell lines (with $IC_{50} < 100$ nmol/L) are SCLC in origin. Non–small cell lung cancer (NSCLC) cell lines are less sensitive than SCLC cells and the potency of JQ1 in inhibiting the NSCLC lines is comparable with results reported previously (12, 14). The differential sensitivity of SCLC cell lines

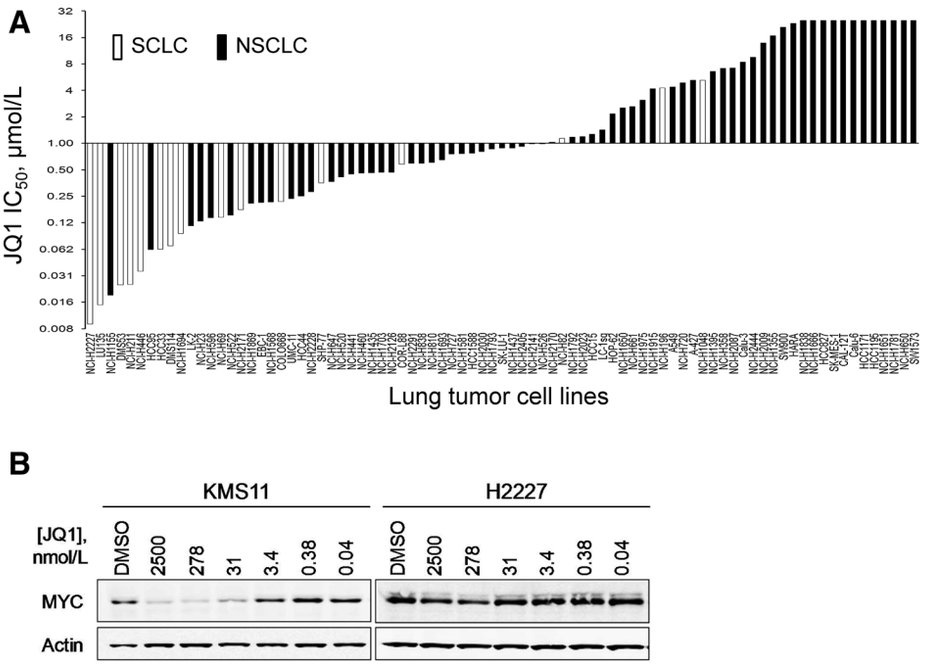

Figure 1.
SCLC cells are sensitive to BET inhibition. A, JQ1 IC_{50} in cell proliferation assays with a panel of lung cancer cell lines. Data shown are the average of results from triplicate experiments. The histologic type of the cell lines is as indicated. B, effect of JQ1 treatment on MYC protein in a multiple myeloma (KMS11) and a SCLC (H2227) cell line. Cells were treated for 2 hours, and whole-cell lysates were analyzed by Western blotting with antibodies to MYC or actin.

was also observed when a subset of the lung tumor cell lines was tested with another BET inhibitor I-BET762 (Supplementary Fig. S1; ref. 5). In previous studies using multiple myeloma and AML cell lines, JQ1 was found to inhibit cell proliferation by downregulating the expression of *MYC* (6, 7, 9). *MYC* is highly expressed in the sensitive SCLC cell line H2227, but JQ1 had only a slight effect on MYC protein, whereas in myeloma cell line KMS11, MYC expression was downregulated by >80% (Fig. 1B). Among the SCLC lines, there is no apparent relationship between JQ1 sensitivity and mutation or amplification of the major cancer genes, such as *RB1*, *STK11*, *TP53*, or *MYC* (Supplementary Table S1; ref. 23). JQ1 appears to be cytotoxic to lung tumor cells that are highly sensitive, as evident in the loss of cells compared with untreated cultures (Supplementary Fig. S2). The mechanism of growth inhibition of SCLC lines by JQ1 was further examined in a caspase-3/7 cleavage assay, performed with a subset of resistant and sensitive SCLC cell lines. Upon treatment with JQ1, there was a dose-dependent increase in caspase-3/7 cleavage in the sensitive cell lines (H2227, LU135, and DMS53), but not in the resistant cell line H1048 (Supplementary Fig. S3). Together, these data indicate that SCLC cells are selectively sensitive to growth inhibition by JQ1 through a mechanism that involves apoptosis.

JQ1 regulates *ASCL1* expression in SCLC

A transcription profiling study was undertaken to investigate the mechanistic basis of the sensitivity of SCLC cells to JQ1. The goal was to identify gene-expression changes that may account for the differential sensitivity of the SCLC cell lines to JQ1. Four SCLC cell lines were treated with JQ1 at 12 concentrations (0.04 nmol/L to 7.5 μmol/L) for 2 hours and mRNA was isolated and subjected to microarray analyses. The cell lines used included two that are highly sensitive (DMS53 and H2227), one moderately sensitive (H1694), and one resistant (H1048) to JQ1. The treatment time was limited to 2 hours to minimize detection of expression changes that are secondary or indirect. The SDRS algorithm (19) was applied to the microarray data, and it identified probe sets that show dose-dependent regulation by JQ1. The probe sets were filtered for those that showed ≥2-fold change with EC_{50} < 750 nmol/L in at least one cell line, with a statistical cutoff of $P < 0.05$. Of 1537 probe sets that met the criteria, the majority were regulated in all cell lines, and hence not included for further analysis because they are not likely to account for the differential sensitivity of the cell lines to JQ1. However, a subset of these changes are noteworthy because they may reflect the transcription dynamics affected by JQ1 that are not manifested in the physiologic response. In all four cell lines, JQ1 upregulated the expression of *HEXIM1*, *IER5*, and *STK17A* (Supplementary Fig. S4). HEXIM1 is an inhibitor of the cdk9–cyclin T1 complex for RNA polymerase II transcript elongation, and has been shown to be induced by JQ1 (24, 25). IER5 and STK17A are proteins that have been implicated in radiation-induced cell-cycle checkpoint and in apoptosis, respectively (26, 27). The upregulation of these genes may be necessary, but not sufficient for the antiproliferative effects of JQ1, although further work will be required to establish this functional assignment to the proteins. In previous studies of JQ1 sensitivity in NSCLC cells, *FOSL1* modulation was proposed to mediate the inhibition of NSCLC cells by JQ1 (12). *FOSL1* mRNA is expressed at low or undetectable levels in SCLC cell lines and is not likely to play a role in JQ1 sensitivity in SCLC.

The microarray data analyses identified 53 probe sets, representing 47 genes, that showed differential response to JQ1 across the cell lines (Fig. 2A) Further examination of the dose–response data revealed that many of these probe sets were expressed at background levels in all cell lines (e.g., RNF183) or in the sensitive cell lines (e.g., NR0B2; Supplementary Table S2 and Supplementary Fig. S5) Only one gene, *ASCL1*, is differentially expressed in the sensitive cell lines, and was downregulated by 2.3- to 5-fold with JQ1 treatment (Supplementary Fig. S6). *ASCL1* encodes a transcription factor that is expressed in developing neurons and in neuroendocrine lung

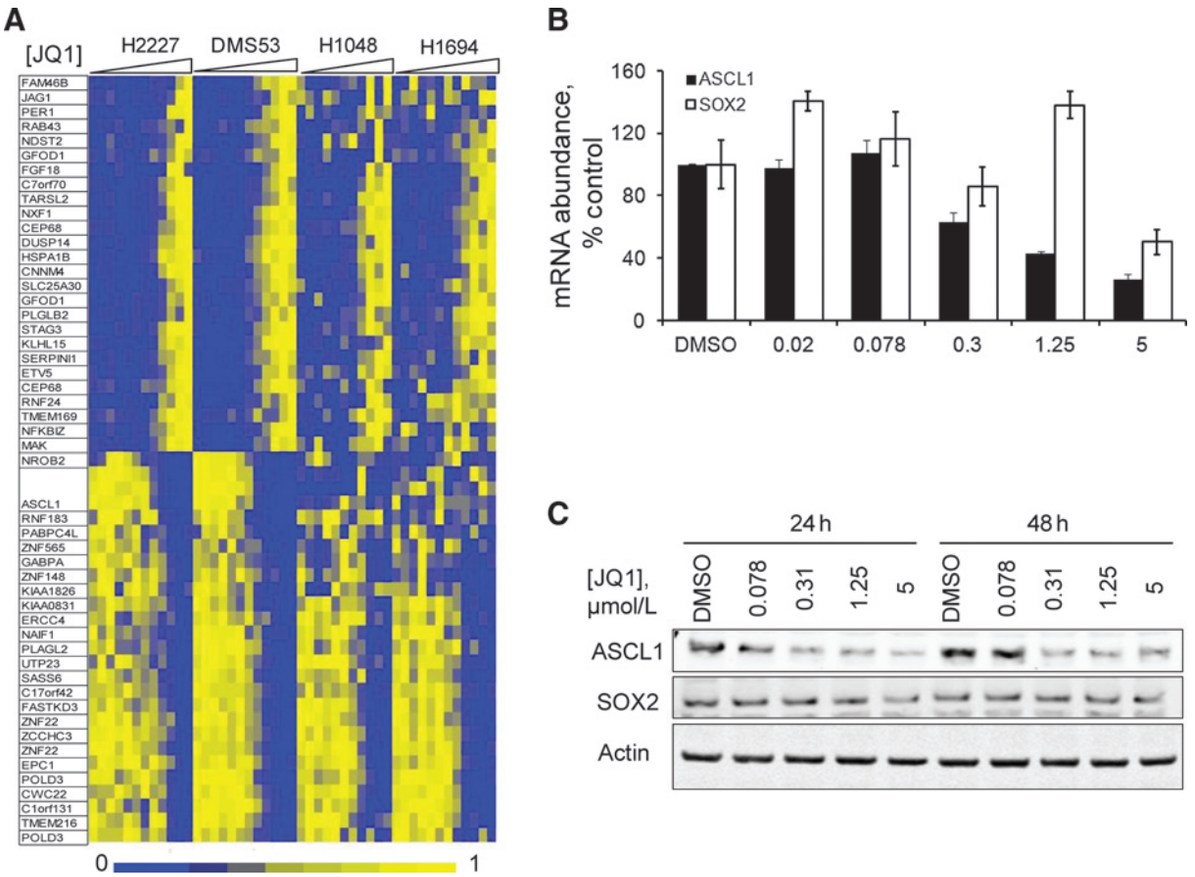

Figure 2.
Transcriptional response to BET inhibition in SCLC cell lines. A, relative expression intensity of 53 probe sets across a dose range of JQ1 treatment in four cell lines. These probe sets met the criterion of showing dose response in only the two sensitive lines. Signal intensity was scaled from 0 (blue) to 1 (yellow). B, DMS53 cells were treated with JQ1 for 4 hours and *ASCL1* and *SOX2* transcript levels were measured by qPCR. Data shown are averages of triplicate determinations. C, Western blot analyses of ASCL1 and SOX2 proteins after 24 and 48 hours of JQ1 treatment in DMS53 cells.

cancer (16, 17). The regulation of *ASCL1* expression in SCLC cell lines that are highly sensitive to JQ1 suggests that *ASCL1* may be important to the proliferation and/or survival of SCLC cells. The dose-dependent modulation of *ASCL1* expression by JQ1 was confirmed in DMS53 cells by quantitative PCR analysis (Fig. 2B). *SOX2*, another transcription factor previously implicated in lung cancer (28), is also highly expressed in DMS53 cells, but was only modestly affected at the highest concentration of JQ1 tested (Fig. 2B). Analyses of ASCL1 and SOX2 proteins further confirmed the selective modulation of *ASCL1* expression by JQ1 (Fig. 2C). These data suggest that JQ1 regulates gene expression in SCLC in a highly specific manner, and support further investigation into the role of *ASCL1* as the mechanism of action mediating JQ1 sensitivity in SCLC.

BRD4 binds directly to the *ASCL1* gene and enhancer

We further investigated the mechanism whereby JQ1 regulates *ASCL1* expression. DMS53 cells were treated with JQ1 for 4 hours, and the compound was removed from the culture medium. *ASCL1* expression was inhibited by >80% with the 4 hours treatment, but recovered to close to 80% of the original level by 60 minutes after removal of the compound (Fig. 3A). The rapid decrease and recovery of *ASCL1* expression suggest that the *ASCL1* gene may be a direct target of the BET proteins BRD2/BRD4, which have been shown to mediate transcription regulation by JQ1 in other cancer cell lines (4–9). The direct binding of BRD4 to the *ASCL1* gene was confirmed by chromatin immunoprecipitation using an antibody to BRD4. BRD4 is highly enriched at the *ASCL1* enhancer, compared with a gene desert area on the same chromosome (Supplementary Fig. S7A). In both DMS53 and H2227 cells there is high density of BRD4 and RNA polymerase II binding at the *ASCL1* gene, consistent with *ASCL1* being an actively transcribed gene in these cells and with BRD4 being a pivotal mediator of its transcription (Fig. 3B and C). JQ1 treatment resulted in a significant reduction in BRD4 and RNA polymerase II peak intensity in both cell lines (Fig. 3B and C). There is significant enrichment of BRD4 binding at the *ASCL1* enhancer in both DMS53 and H2227 cells, which is reduced upon JQ1 treatment (Fig. 3D and E). By contrast, background levels of BRD4 binding to the genome were not modulated by JQ1 treatment (Supplementary Fig. S7B). These data, therefore, support the conclusion that BRD4 regulates *ASCL1* expression by direct binding to its enhancer.

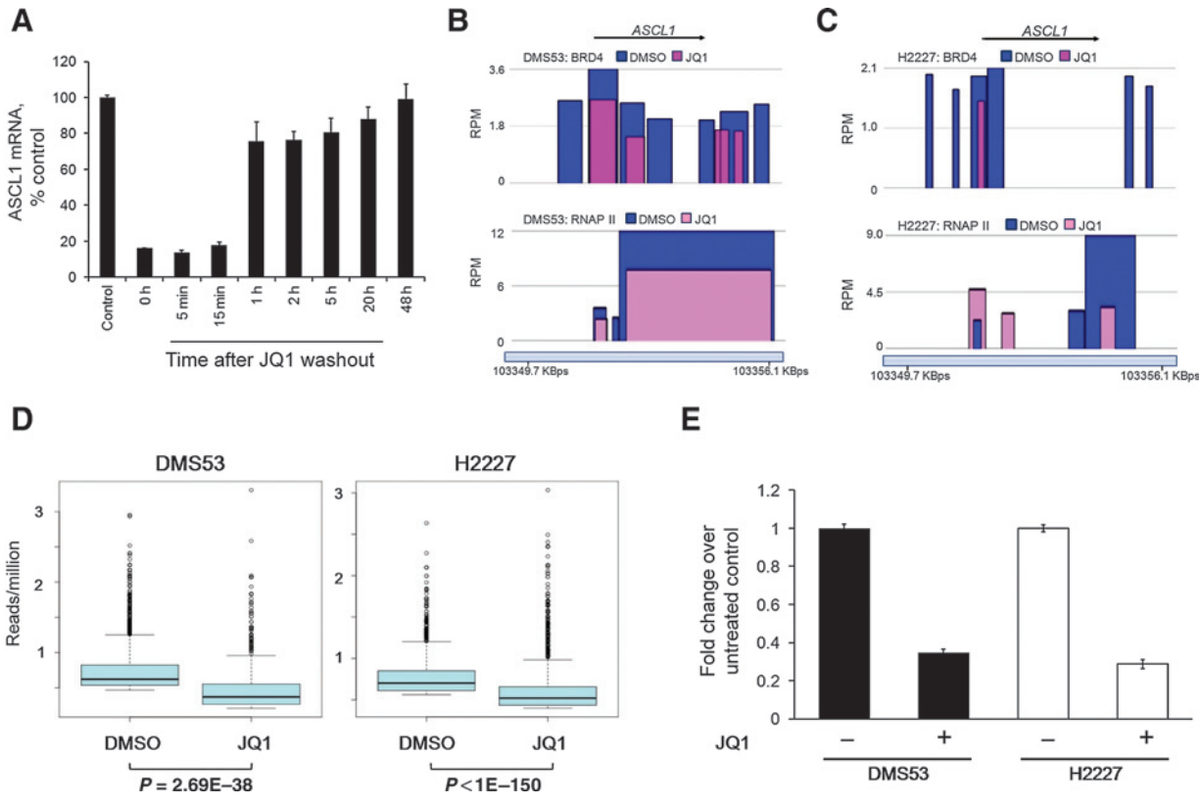

Figure 3.

JQ1 antagonizes the binding of BRD4 to *ASCL1* gene and enhancer. A, recovery of *ASCL1* expression in DMS53 cells following JQ1 treatment and washout. DMS53 cells were treated with 5 μmol/L JQ1 for 4 hours, and the drug treatment was terminated by replacing the culture medium with fresh medium without drug. *ASCL1* expression was measured by qPCR, at different times following the medium replacement. B and C, effect of JQ1 on BRD4 and RNA polymerase II binding at the *ASCL1* gene of DMS53 and H2227 cells. Shown are CHIP-seq peaks distributed across the *ASCL1* gene in the two cell lines. Peaks are filtered at $P < 0.01$ against input and the peaks for both DMSO and JQ1 treatments are overlaid. Data represent the averages of triplicate experiments. D, in both cell lines, the global average of the reads per million (RPM) per base pair was significantly lower in samples from cells treated with JQ1 compared with those from cells treated with DMSO only. E, displacement of BRD4 binding at the *ASCL1* enhancer is observed upon treatment with JQ1 at 500 nmol/L for 4 hours compared with DMSO control.

ASCL1 is required for growth of SCLC cells sensitive to JQ1 and is expressed at high levels in SCLC and lung neuroendocrine cancer cells

The importance of *ASCL1* to SCLC was further investigated using siRNA knockdown. Pooled and four individual siRNAs targeting *ASCL1* were transiently transfected into DMS53 cells, and downregulation of *ASCL1* expression was confirmed by Western blot analysis. The *ASCL1* siRNA pool, as well as the individual siRNA all reduced ASCL1 protein to different extent, relative to the level in cells transfected with a siRNA with a scrambled sequence (Fig. 4A). The maximum reduction in ASCL1 protein achieved with siRNA was comparable with that attained with JQ1. The *ASCL1* siRNA treatment reduced viability of DMS53 cells, to an extent that is commensurate with the extent of ASCL1 protein reduction (Fig. 4B). By contrast, the *ASCL1* siRNA had no effect on the viability of H1048 cells, which do not express ASCL1 and are resistant to JQ1. Both cell lines are sensitive to knockdown of KIF11, a kinesin that is an essential gene in all dividing cells (21), thus ensuring that the differential sensitivity to ASCL1 knockdown is not attributable to differences in transfection efficiency (Fig. 4B). There is a clear correlation between *ASCL1* expression and JQ1 sensitivity in the SCLC cell lines (Supplementary Fig. S8A and S8B). It is noteworthy that two of the NSCLC cell lines (NCI-H1155 and UMC-11) that are sensitive to JQ1 also have relatively high *ASCL1* expression (Supplementary Fig. S8A). These two NSCLC cell lines are documented to be neuroendocrine in histology, and their *ASCL1* mRNA abundance is consistent with the suggestion that *ASCL1* is a lineage-specific marker for lung tumor cells with neuroendocrine features (18, 29). Treatment of the neuroendocrine cell line UMC-11 with JQ1 also resulted in reduction of *ASCL1* expression (Supplementary Fig. S8C).

Results of these studies highlighted the importance of *ASCL1* expression to JQ1 sensitivity in SCLC cell lines, and a potential role of *ASCL1* as a driver oncogene. These observations prompted us to examine the expression of *ASCL1* in tumor biopsies obtained from SCLC patients. Analyses of RNA-seq data from a published SCLC genome study (30) revealed that *ASCL1* is highly expressed in >50% of patients, at a level that is considerably higher than that of *SOX2* (Fig. 5). In these clinical samples, expression of *MYC* and *MYCN* is elevated only in a relatively small number of patients, suggesting a lesser role for these two oncogenes in SCLC.

Discussion

The BET proteins have been shown to promote active gene expression by recruiting other factors that facilitate RNA

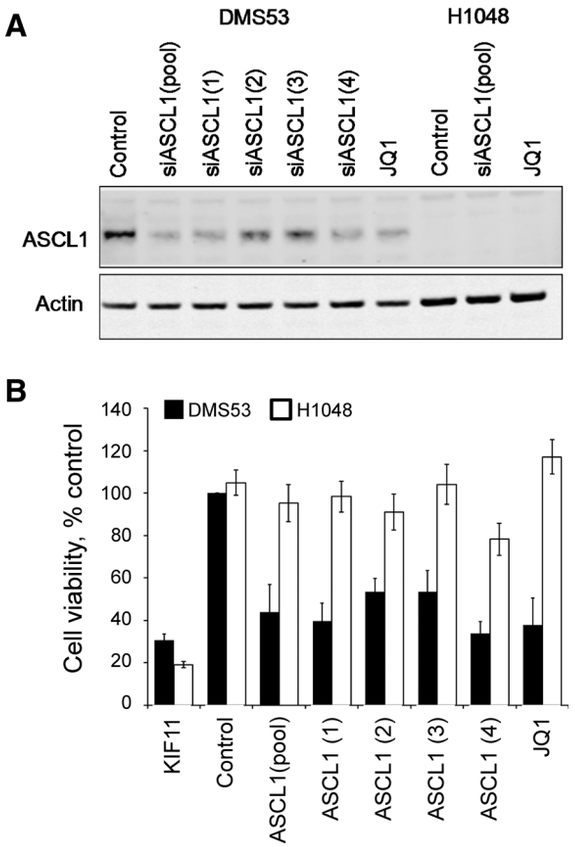

Figure 4.
ASCL1 is required for SCLC cell viability. A, Western blot analysis of DMS53 cell lysates after treatment with siRNA or JQ1. Cells were transfected with *ASCL1* pooled or individual siRNA, or a control siRNA with scrambled sequence. Cell lysates were harvested after 48 hours treatment with siRNA or 24 hours treatment with JQ1 (500 nmol/L). B, viability of DMS53 and H1048 cells after 72 hours treatment with *ASCL1* siRNA or JQ1.

polymerase II pause release. A role for the BET proteins in driving expression of *MYC*, important to the cancer phenotype in subsets of myeloma and leukemic cells, was revealed by means of the small-molecule JQ1. The discovery of JQ1 and other potent and selective small molecules targeting the BET proteins has provided proof of concept that chromatin regulators can be tractable drug targets. Two previous studies reported that subsets of lung cancer cell lines are sensitive to JQ1, although their analyses were restricted to cells of non–small cell histology (12, 14). In this study, a comprehensive survey of lung tumor cell lines was performed with JQ1, and resulted in the novel finding that SCLC cells are comparatively more sensitive to JQ1 than those of non–small cell lung histology. Transcription profiling studies performed in sensitive and resistant SCLC cells revealed that JQ1 treatment resulted in dose-dependent up- or downregulation of approximately 1,500 probe sets within 2 hours. More than 95% of these probe sets were changed in all cell lines and are not likely to be linked to the differential sensitivity of SCLC cells to growth inhibition by JQ1. These changes may reflect the global role of BET proteins in regulating the expression of genes required for cell-cycle progression (31), and account for the low level of JQ1 sensitivity across the majority of the lung cell lines. Of 47 genes that were regulated only in sensitive cell lines, only one gene, *ASCL1*, has the expression and regulation pattern that highly correlates with the growth inhibition by JQ1.

ASCL1 encodes a basic helix-loop-helix transcription factor that is essential for the development of lung neuroendocrine cells (16). Overexpression of *ASCL1* transcript was previously reported in SCLC and in lung tumors of neuroendocrine origin (17, 18). We have shown that *ASCL1* is highly expressed in SCLC cell lines that are sensitive to JQ1, and JQ1 treatment results in a rapid and reversible inhibition of *ASCL1* gene expression. Our CHIP-seq data show a high density of BRD4 at the *ASCL1* gene, and suggest that BRD4-containing transcriptional complexes at the ASCL1 enhancer are the direct target of JQ1. Knockdown of ASCL1 in the SCLC cell line DMS53 resulted in inhibition of cell proliferation, providing additional support for a critical function of ASCL1 in SCLC. Our observation with DMS53 cells is consistent with previous reports that lung cancer cells with *ASCL1* upregulation are sensitive to ASCL1 siRNA knockdown (18, 32). Our results have, therefore, provided further validation of ASCL1 as a driver of proliferation in SCLC. Although ASCL1 has been established as a proneural transcription factor that drives neuronal differentiation, it has also been found to drive the proliferation of neural

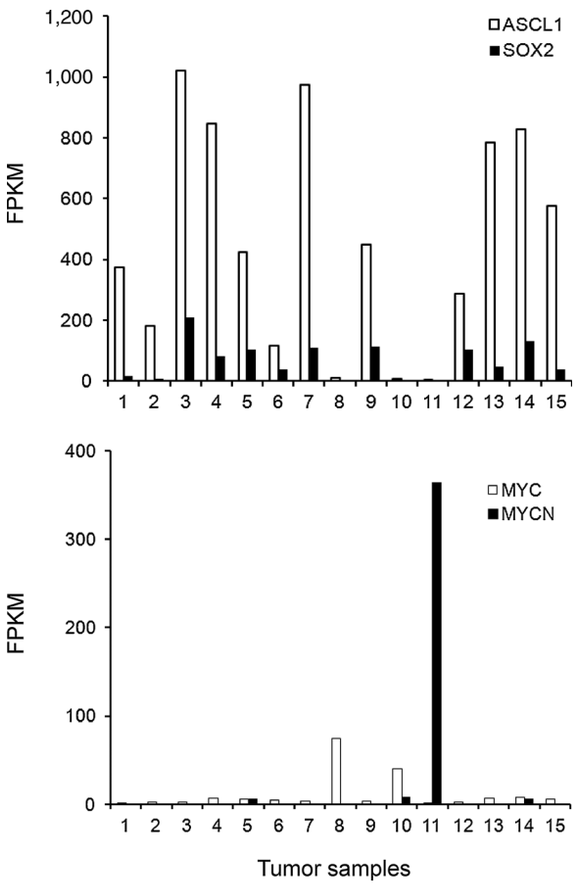

Figure 5.
Overexpression of *ASCL1* in SCLC clinical specimens. RNA-seq data from Peifer et al. (26) were analyzed to compare the expression of *ASCL1* and *SOX2* (top), as well as that of *MYC* and *MYCN*. Transcript abundance was reported as fragments per kilobase per million reads (FPKM).

progenitor cells by positive regulation of such genes as *CDK2*, *E2F1*, and *BIRC5* (33). The proposed function of ASCL1 as an oncogenic transcription factor is, therefore, supported by its known function in upregulating growth promoting genes.

The characteristics of SCLC genomes include a high prevalence of mutations in the tumor suppressors *RB1* and *TP53*. More recently, whole-genome sequencing of SCLC specimens have uncovered potential driver mutations such as *PTEN* mutation or *FGFR1* amplification, both of which occur at relatively low frequency (28, 30). In one previous study, *SOX2* amplification was found in approximately 27% of SCLC samples, and *SOX2* knockdown by shRNA inhibited proliferation of SCLC cells with *SOX2* gene amplification (30). We analyzed the RNA-seq data published in that report, and found that *ASCL1* is expressed at high levels in 10 of 15 SCLC specimens, to a level greater than *SOX2*. These findings further underscore the importance of *ASCL1* as a potential driver oncogene in SCLC. More importantly, our results have demonstrated that inhibition of BET protein binding to chromatin is a feasible approach for pharmacologic inhibition of *ASCL1* expression and consequently SCLC cell growth. Our findings do not preclude a role for *SOX2* in SCLC, but a pharmacologic approach to inhibit *SOX2* expression remains to be demonstrated. Furthermore, the RNAseq data point to a higher prevalence of *ASCL1* deregulation in the disease and perhaps a greater therapeutic opportunity using BET antagonists. Inhibition of CDK7 was recently shown to modulate the expression of genes associated with superenhancer features in SCLC (34). These highly expressed genes, including *ASCL1*, are enriched in those encoding transcription factors specific to a neuroendocrine lineage, and their modulation was proposed to mediate the sensitivity of SCLC cell lines to CDK7 inhibition. These recent findings are consistent with our proposal that *ASCL1* has an important role in the survival of SCLC. BET and CDK7 inhibitors are, therefore, two independent pharmacologic approaches for modulating the expression of putative oncogenic transcription factors, and their relative effectiveness will have to be further assessed in preclinical and clinical testing.

SCLC is a disease with high unmet medical need, with chemotherapy being the only treatment option with a high rate of relapse. This study has provided the basis for evaluating BET inhibitors as a novel treatment option for SCLC. Furthermore, our data suggest that *ASCL1* expression may be used to select lung cancer patients who are likely to respond to BET inhibitor treatment.

Disclosure of Potential Conflicts of Interest

C. Fairchild has ownership interest (including patents) in BMS stock. No potential conflicts of interest were disclosed by the other authors.

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R. Lenhart, S. Kirov, H. Desilva, L. Schweizer, P. Ross-Macdonald, C. Fairchild, T. Wong, S. Wee
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