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Targeted reduction of KLF6-SV1 restores chemotherapy sensitivity in resistant lung adenocarcinoma

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Abstract

Kruppel-like factor 6 splice variant 1 (KLF6-SV1) is an oncogenic splice variant of the KLF6 tumor suppressor gene that is specifically overexpressed in a number of human cancers. Previously, we have demonstrated that increased expression of KLF6-SV1 is associated with decreased survival in lung adenocarcinoma patient samples and that targeted reduction of KLF6-SV1 using siRNA induced apoptosis both alone and in combination with the chemotherapeutic drug cisplatin. Here, we demonstrate that chemoresistant lung cancer cells express increased levels of KLF6-SV1. Furthermore, targeted reduction of KLF6-SV1 using RNA interference restores chemotherapy sensitivity to lung cancer cells both in culture and in vivo through induction of apoptosis. Conversely, overexpression of KLF6-SV1 resulted in a marked reduction in chemotherapy sensitivity in a tumor xenograft model. Combined, these findings highlight a functional role for the KLF6-SV1 splice variant in the regulation of chemotherapy response in lung cancer and could provide novel insight into lung cancer therapy.

INTRODUCTION

Lung cancer is a leading cause of caner death in the United States, with over 162,460 deaths from lung cancer in the US exceeding cancer mortality from colorectal, breast, prostate, and pancreatic cancer combined (1). The aggregate survival benefit from use of chemotherapy in the treatment of lung cancer is supported by evidence from dozens of randomized controlled trials (2). Though the data supports a benefit in large groups of patients, variability in individual response to chemotherapy and the development of resistance after either discontinuation of treatment or during treatment itself is a major cause of patient morbidity and mortality (2,3). Treatment failure results from the development of resistance to chemotherapy and is characterized by the selection for cancer cells with either defects in the apoptotic cascade, increased expression of nucleotide excision repair pathway members, and/or increased activity of drug efflux transporters (4,5). Targeted therapy to the specific molecular alterations underlying the development of chemotherapy resistance represents an appealing therapeutic strategy.

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KLF6 (Zf9/CPBP) (GeneBank accession number AF001461) is a member of the Krüppel-like factor (KLF) family, that was originally shown to be functionally inactivated by loss of heterozygosity (LOH) and somatic mutation in sporadic prostate adenocarcinomas (6). More recent reports have extended the range of human tumors and the mechanisms by which KLF6 can be inactivated to include deletion of the KLF6 locus and mutation in colorectal cancers (7), hepatocellular and gastric carcinomas (8,9), LOH in ovarian carcinoma and gliobastoma (10,11), decreased KLF6 expression in non-small cell lung cancer (12,13), hypermethylation of the promoter region in esophageal SCC cell lines and hepatocellular carcinoma patient samples (14,15). In addition, three alternatively spliced KLF6 isoforms have been identified (16) and at least one of them, KLF6-SV1, has been shown to be biologically active, antagonizing the tumor suppressor function of KLF6 and promoting tumor growth and dissemination (10,16,17).

A role of KLF6 in non-small cell lung cancer (NSCLC) was first suggested by microarray studies comparing gene expression between normal and lung cancer specimens (18). COPEB/KLF6 was decreased in malignant compared to benign lung tissue but high KLF6 expression levels in tumor specimens were associated with advanced disease stages and contributed to a prognostic gene signature of poor survival (18). Furthermore, recent reports suggest that decreased KLF6 expression is a common event in lung adenocarcinoma and overexpression of KLF6 in lung cancer cell lines induced spontaneous apoptosis (13). Previously, we have demonstrated that increased expression of KLF6-SV1 is associated with decreased survival in lung adenocarcinoma patient samples and that targeted reduction of KLF6-SV1 using siRNA induced apoptosis both alone and in combination with the chemotherapeutic drug cisplatin (19). Given the evidence for a role for the KLF6 tumor suppressor gene in lung cancer (12, 13,19), we sought to define the role of the oncogenic splice variant, KLF6-SV1 in the development of chemotherapy resistance and whether targeted reduction of KLF6-SV1 could restore chemotherapy sensitivity in chemoresistant lung cancer cell lines.

MATERIALS AND METHODS

Cell culture and cell line generation

All cell lines were obtained from the American Tissue Culture Collection (ATCC). Retroviral infection with KLF6-SV1 was performed as previously described (10,21,22). The A549 resistant cell lines were generated by serial selection in cisplatin (final concentration 20 μM) for 2 passages for the A549-IR cell line and 8 passages for the A549-CR cell line. Transient transfection of a non-targeting control and SV1 siRNA was performed with Lipofectamine 2000 (Invitrogen) in the A549 lung cancer cell lines as previously described (21,22). Briefly, 50,000 cells per 12 well dish were plated for each cell line. Cells were then transfected with equal amount of the siRNA and harvested at 72hr for RNA, protein, and FACS analysis. For chemotherapy experiments, cisplatin (Sicor Laboratories) was added to a final concentration of 20 μM 24hrs after either plating of the A549-P, A549-IR, or A549-CR stable cell lines or transfection with si-NTC or si-SV1 in all lung cancer cell line studied.

Western Blot Analysis

Cell extracts for Western blotting were harvested in radioimmunoprecipitation assay buffer (standard protocols, Santa Cruz Biotechnology). Tumor tissue extracts were harvested and prepared in the T-PER reagent (Pierce, Rockford, IL). Equal amounts of protein (50 μg) as determined by the Bio-Rad (Richmond, CA) DC Protein quantification assay were loaded and separated by PAGE and transferred to nitrocellulose membranes. Western blotting was done using a goat polyclonal antibody to Actin, rabbit polyclonal antibodies to NOXA, MCL1, Caspase 3, 8, and 9 (Cell Signalling Technology), and monoclonal antibodies to KLF6 2A2 (Zymed).

RNA and qRT-PCR Analysis

Cell line and tumor RNA was extracted using the Rneasy Mini and Midi kit (Qiagen). All RNA was treated with DNAse (Qiagen). A total of 1μg of RNA was reverse transcribed per reaction using first strand complementary DNA synthesis with random primers (Promega). qRT-PCR was performed using the PCR primers previously described (10,11,21) on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). All experiments were done in triplicate and independently validated three times. All values were normalized to GAPDH levels. All primer sequences and methods for quantifying KLF6 alternative splicing were performed as previously described (10,11,21,22).

FACS Analysis

Cells were harvested from 12 well dishes and fixed in absolute ethanol (Sigma) for 24 hours. On the day of analysis, cells were pelleted and the absolute ethanol was removed. Cells were then stained with a 1mL solution containing propidium iodide (PI), RNase A, and PBS. FACS analysis was performed on the FACSCalibur instrument (BD Biosciences).

Tumorigenicity assay

The parental and cisplatin resistant A549 cell lines (A549-NR and A549-CR) (1×10^6) or the A549-pBABE and SV1 stable cell lines were injected into the left flank of 6- to 8-week-old female BALB/c nu/nu mice. Tumor volume was assessed every week and determined by the formula (length × width × width × 0.4). Three weeks after injection of the tumor cells, mice were treated with intraperitoneal cisplatin at a concentration of 5mg/kg twice a week for three weeks. Tumor volumes were determined prior to each injection. Treatment response was defined as a decrease in tumor size of greater than 50%. The mice were sacrificed 8 weeks and tumors were excised for RNA, protein, and immunohistochemical analysis. All animal work and protocols were approved by the Mount Sinai School of Medicine Institutional Animal Care and Use Committee.

RESULTS

KLF6-SV1 expression is increased in a chemotherapy resistant lung cancer cell line

The A549 lung adenocarcinoma cell line was serially selected in media containing chemotherapy for between 2 and 8 passages. Two derivative cell lines were generated after selection that were then tested for their sensitivity to cisplatin. While the addition of cisplatin to the parental A549 cell line (A549-NR) resulted in significant induction of apoptosis, a marked reduction in apoptosis was seen in both the A549 cisplatin resistant cell line (A549-CR) and the A549 Intermediate Resistant cell line (A549-IR) (Figure 1a,b) (*** $p < 0.001$). Genotype analysis of 10 distinct polymorphic regions at 8 different chromosomal regions revealed that these chemotherapy resistant cell lines were clonally derived from the parental A549 cell line (data not shown). There were no differences in cellular proliferation as assessed by triatiated thymidine incorporation between the chemoresistant and parental cell lines. In order to determine the role of the oncogenic splice variant KLF6-SV1, quantitative real time PCR (qRT-PCR) and western blot analysis for expression of KLF6-SV1 was performed (10, 11,19). There was a significant increase in KLF6-SV1 expression at both the mRNA and protein level in the both the A549-IR and A549-CR chemoresistant cell line when compared to control (Fig. 1c,d and data not shown) (** $p < 0.01$). This change in KLF6-SV1 expression was associated with decreased expression of the cyclin dependent kinase inhibitor p21, a known transcriptional of the wtKLF6 tumor suppressor gene, at both the mRNA and protein level (Figure 1d). In addition, the ratio of KLF6-SV1 to wtKLF6 was significantly increased in the A549 chemoresistant cell lines compared to the parental cell line (Figure 1e) (** $p < 0.001$). Together, this data suggests that acquired chemotherapy resistance is associated with increased

expression of KLF6-SV1 and that this change may be drive the phenotypic conversion of chemosensitive cells to their resistant counterparts.

Targeted reduction of KLF6-SV1 expression restores chemotherapy sensitivity in the A549-CR cell line

To determine the biological and functional relevance of increased KLF6-SV1 expression in chemoresistant lung cancer cell lines, we next sought to directly target KLF6-SV1 using RNA interference (RNAi). Using chemically modified siRNA specific to KLF6-SV1 as previously described (10,21,22), we explored the possibility that targeted reduction of KLF6-SV1 could restore chemotherapy sensitivity in the resistant lung adenocarcinoma cell line, A549-CR. Transient transfection of KLF6-SV1 specific siRNA resulted in efficient silencing of KLF6-SV1 expression at both the RNA and protein level at 72 post-transfection with no effect on the expression of other KLF6 isoforms (Fig 2a, data not shown) (*** $p < 0.001$). Targeted reduction of KLF6-SV1 alone resulted in increased in spontaneous apoptosis in the cisplatin resistant cell line (A549-CR) as shown (Fig 2b) (*** $p < 0.001$). In addition, combination treatment with cisplatin and si-SV1 resulted in increased apoptosis in this highly chemoresistant cell line. Previous reports demonstrated that targeted reduction of KLF6-SV1 results in marked activation of the intrinsic pathway of apoptosis through upregulation of the pro-apoptotic NOXA and degradation of the anti-apoptotic Mcl-1 (19,21,22). To determine if the si-SV1 induced apoptosis in the chemoresistant A549-CR cell line was mediated by NOXA and Mcl-1 we analyzed the expression of both NOXA and MCL-1 at the mRNA and protein level. As shown in Figure 2c, treatment of the A549-CR with both siRNA to KLF6-SV1 and cisplatin resulted in a marked increase in NOXA expression (Figure 2c, d, and data not shown). In addition, we also analyzed the expression and activation of Caspase 3 and 8. Addition of cisplatin with KLF6-SV1 inhibition led to a significant increase in the amount of all active caspases (Fig 2d). Moreover, the combination of siSV1 and cisplatin resulted in the greatest degree of caspase activation (Fig 2d). Together this data suggests that targeted downregulation of KLF6-SV1 using RNAi in combination with cisplatin results in spontaneous apoptosis in chemoresistant lung cancer cell lines.

Increased KLF6-SV1 expression results in chemotherapy resistance in vivo

To extend these findings to an *in vivo* model of chemotherapy resistance, we injected both the parental A549 cell line and A549-CR line in immunodeficient nude mice. Tumors derived from either the parental or resistant cell lines were allowed to form subcutaneously for three weeks and tumor volume was then determined prior to treatment with cisplatin. Intraperitoneal injection of cisplatin was performed at a concentration of 5mg/kg twice a week for three weeks, tumor volumes were determined prior to each injection and mice were then sacrificed at the end of the treatment period (Fig. 3a). The starting volumes of the tumors was not significantly different (Figure 3b). Treatment response was defined as a decrease in tumor size of greater than 50%. As shown in Figure 3c, 62% of the parental A549 cells responded to cisplatin in vivo as compared to only 16% of the A549 resistant cell line derived tumors (* $p < 0.01$). At the end of the treatment period, the A549-NR derived tumors were 40% smaller than the A549-CR tumors (*** $p < 0.001$). KLF6-SV1 expression was significantly higher in the resistant tumors compared to tumors derived from the parental cell line (Fig. 3c) (*** $p < 0.001$). Interestingly, KLF6-SV1 levels were significantly higher at both the mRNA and protein level in parental A549 tumors that were resistant to chemotherapy compared to tumors that were chemosensitive (Fig. 3c) (** $p < 0.01$). Western blot analysis of the tumors derived from the A549 chemosensitive and resistant cells revealed a marked decrease in expression of the proapoptotic NOXA with concimitant increase in the expression of MCL1 (Fig 3c). Combined, this data suggests that lung cancer chemoresistance is associated with increased expression of KLF6-SV1 which results dysregulated expression of both pro- and antiapoptotic genes in vivo.

To further characterize the role of KLF6-SV1 in cisplatin resistance *in vivo*, we generated stable cell lines overexpressing KLF6-SV1 in the A549 lung cancer cell line as previously described (19). Retroviral infection of this cell line resulted in a 10-fold overexpression of KLF6-SV1 mRNA and a 5-fold change in protein expression when compared to pBABE infected control cells (Fig 4a). The pBABE and SV1 stable cell lines were then injected subcutaneously into immunodeficent mice. Tumors derived from either the parental or SV1 cell lines were allowed to form for three weeks and tumor volume was then determined prior to treatment with cisplatin. Intraperitoneal injection of cisplatin was performed at a concentration of 5mg/kg twice a week for three weeks, tumor volumes were determined prior to each injection and mice were then sacrificed at the end of the treatment period. Treatment response was defined as a decrease in tumor size of greater than 50%. As shown in Figure 4b, 87.5% of the pBABE A549 cells responded to cisplatin *in vivo* as compared to only 11% of the SV1 A549 derived tumors (Fig. 4b) (* $p < 0.01$). There was no significant difference in the average starting volumes of the tumors between the two groups (Figure 4b). On average, KLF6-SV1 derived tumors showed no reduction in tumor volume after treatment with cisplatin versus a 35% reduction in average tumor volume in the A549-pBABE treated tumors (Figure 4B). KLF6-SV1 expression was significantly higher in the resistant tumors compared to parental cell line derived tumors (data not shown). Western blot analysis of the tumors derived from both the pBABE and SV1 A549 cells revealed a marked increase in the expression of MCL1 (Fig 4c) and concomitant downregulation of NOXA in these tumors. Combined this data suggests that overexpression of KLF6-SV1 results in decreased sensitivity to cisplatin *in vivo* through regulation of NOXA and Mcl-1 expression.

DISCUSSION

Lung cancer is the leading cause of cancer-related death in the United States. While early stage disease is treatable with a 5 yr survival exceeding 70%, the prognosis of patients with metastatic lung cancer remains dismal (4). Treatment failure and the development of chemotherapy resistance are common and contribute significantly to the poor prognosis of patients with advanced disease. A better understanding of the molecular mechanisms underlying the development of resistance will allow for the identification of markers for risk prognostication to better guide treatment decisions and for the development of targeted therapies to overcome resistance. Here we demonstrate that that cisplatin resistant lung adenocarcinoma cells express significantly higher levels of the KLF6-SV1 splice variant. Furthermore, targeted downregulation of this splice variant using siRNA partially restored chemotherapy sensitivity to resistant lung cancer cell lines. Induction of apoptosis in these cell lines was associated with induction of NOXA and activation of caspases 3 and 8. Finally, chemotherapy resistant lung tumors expressed significantly higher levels of KLF6-SV1 and MCL-1 and decreased expression of NOXA consistent with previous reports describing the functional regulation of these pro- and anti-apoptotic molecules by KLF6-SV1 (19,21,22). Overexpression of KLF6-SV1 in tumor xenografts blocked the ability of cisplatin to induce tumor regression. Combined, this data suggests that KLF6-SV1 is an important regulator of chemotherapy resistance in lung adenocarcinoma and that strategies targeting SV1 may provide novel therapeutic opportunities in the treatment of cisplatin resistant lung cancer.

Acknowledgments

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References

1. Jemal A, Murray T, Ward E, et al. Cancer statistics. CA Cancer J Clin 2005;55:10–30. [PubMed: 15661684]
2. Strauss GM. Adjuvant chemotherapy of lung cancer: methodologic issues and therapeutic advances. Hematol Oncol Clin North Am 2005;19:263–81. [PubMed: 15833406]
3. Farray D, Mirkovic N, Albain KS. Multimodality therapy for stage III non small-cell lung cancer. J Clin Oncol 2005;23:3257–69. [PubMed: 15886313]
4. Olaussen KA, Mountzios G, Soria JC. ERCC1 as a risk stratifier in platinum-based chemotherapy for nonsmall-cell lung cancer. Curr Opin Pulm 2007;13:284–9.
5. Modok S, Mellor HR, Callaghan R. Modulation of multidrug resistance efflux pump activity to overcome chemoresistance in cancer. Curr Opin Pharmacol 2006;6:350–4. [PubMed: 16690355]
6. Narla G, Heath KE, Reeves HL, et al. KLF6, a candidate tumor suppressor gene mutated in prostate cancer. Science 2001;294:2563–6. [PubMed: 11752579]
7. Reeves HL, Narla G, Ogunbiyi O, et al. Kruppel-like factor 6 (KLF6) is a tumor-suppressor gene frequently inactivated in colorectal cancer. Gastroenterology 2004;126:1090–103. [PubMed: 15057748]
8. Kremer-Tal S, Reeves HL, Narla G, et al. Frequent inactivation of the tumor suppressor kruppel-like factor 6 (KLF6) in hepatocellular carcinoma. Hepatology 2004;40:1047–52. [PubMed: 15486921]
9. Cho YG, Kim CJ, Park CH, et al. Genetic alterations of the KLF6 gene in gastric cancer. Oncogene 2005;24:4588–90. [PubMed: 15824733]
10. DiFeo A, Narla G, Hirshfeld J, et al. Roles of KLF6 and KLF6-SV1 in ovarian cancer progression and intraperitoneal dissemination. Clin Cancer Res 2006;12:3730–9. [PubMed: 16778100]
11. Jeng YM, Hsu HC. KLF6, a putative tumor suppressor gene, is mutated in astrocytic gliomas. Int J Cancer 2003;105:625–9. [PubMed: 12740910]
12. Kettunen E, Anttila S, Seppanen JK, et al. Differentially expressed genes in nonsmall cell lung cancer: Expression profiling of cancer-related genes in squamous cell lung cancer. Cancer Genet Cytogenet 2004;149:98–106. [PubMed: 15036884]
13. Ito G, Uchiyama M, Kondo M, et al. Kruppel-like factor 6 is frequently down-regulated and induces apoptosis in non-small cell lung cancer cells. Cancer Res 2004;64:3838–43. [PubMed: 15172991]
14. Yamashita K, Upadhyay S, Osada M, et al. Pharmacologic unmasking of epigenetically silenced tumor suppressor genes in esophageal squamous cell carcinoma. Cancer Cell 2002;2:485–95. [PubMed: 12498717]
15. Hirasawa Y, Arai M, Imazeki F, et al. Methylation status of genes upregulated by demethylating agent 5-aza-2′-deoxycytidine in hepatocellular carcinoma. Oncology 2006;71:77–85. [PubMed: 17341888]
16. Narla G, Difeo A, Reeves HL, et al. A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk. Cancer Res 2005;65:1213–22. [PubMed: 15735005]
17. Narla G, DiFeo A, Yao S, et al. Targeted inhibition of the KLF6 splice variant, KLF6 SV1, suppresses prostate cancer cell growth and spread. Cancer Res 2005;65:5761–8. [PubMed: 15994951]
18. Beer DG, Kardia SLR, Huang CC, et al. Gene expression profiles predict survival of patients with lung adenocarcinoma. Nat Med 2002;8:816–824. [PubMed: 12118244]
19. DiFeo A, Feld L, Rodriguez E, et al. A functional role for KLF6-SV1 in lung adenocarcinoma prognosis and chemotherapy response. Cancer Research 2008;68:965–70. [PubMed: 18250346]
20. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene 2006;25:4798–811. [PubMed: 16892092]
21. DiFeo A, Narla G, Huang F, et al. Inhibition of KLF6-SV1 increases ovarian cancer survival through the regulation of NOXA. (submitted).
22. Narla G, DiFeo A, Fernandez Y, et al. KLF6-SV1 is a key regulator of metastasis and survival in prostate cancer. Journal of Clinical Investigation 2008;118:2711–2721. [PubMed: 18596922]

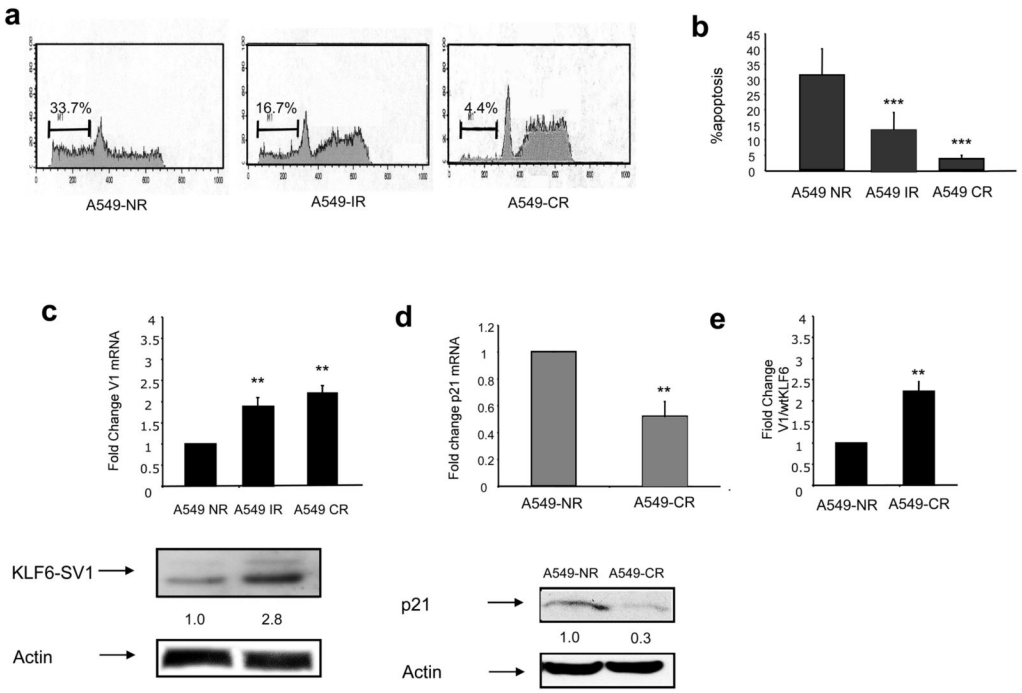

Figure 1.

Expression of KLF6-SV1 in cisplatin resistant lung cancer cell lines. a, Generation and validation of cisplatin resistance in the A549 lung adenocarcinoma cell line. The A549-IR and A549-CR cells were generated by serial selection of the parental A549 cell line in 20 μM cisplatin for 2 and 8 passages respectively. All cell lines were plated at equal densities and treated with 20uM of cisplatin, 72 hrs after treatment cells were harvested. A, Representative FACS analysis of the A549-NR, A549-IR and A549-CR cell line treated with 20 μM cisplatin. FACS analysis of the treated cell lines revealed a marked reduction in the induction of apoptosis in parental A549 cell line (A549-NR) (33.7%) vs. A549-IR (16.7%) vs. A549-CR (4.4%) cell lines. A marked induction of apoptosis is seen in the control A549 cell line while addition of cisplatin results in a G2/M arrest with little induction of apoptosis in the A549-CR cell line. The A549-IR cell line displayed an intermediate sensitivity to cisplatin. b, A significant decrease in apoptosis in the A549-IR and A549-CR compared to the control A549 cell line (*** $p < 0.001$). This was repeated three independent times. C, qtRT-PCR and western blot analysis of the A549-NR, A549-IR, and A549-CR demonstrate a marked upregulation of KLF6-SV1 at both the mRNA and protein level (** $p < 0.001$). d, p21 expression at both the mRNA and protein level in the A549-CR cell line is significantly lower than the parental A549-NR cell line (** $p < 0.001$). e, the ration of KLF6-SV1 to wtKLF6 was determined by quantitative real time PCR (qRT-PCR) using KLF6-SV1 and wtKLF6 specific primers. There is a significant increase in the ratio of KLF6-SV1/wtKLF6 in the cisplatin resistant cell line.

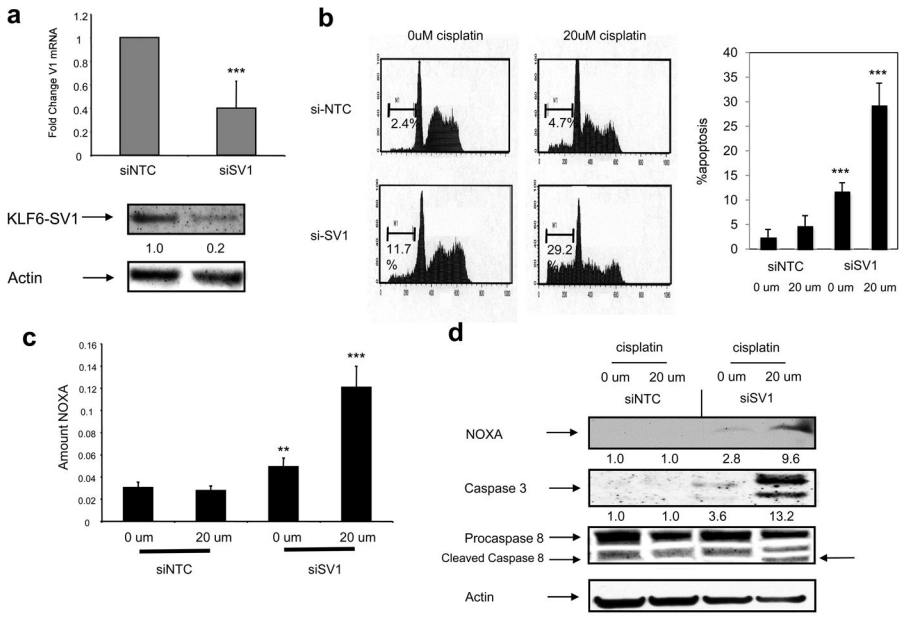

Figure 2.

Targeted reduction of KLF6-SV1 restores chemotherapy sensitivity in cisplatin resistant cell lines. a, qtRT-PCR and western blot analysis of the A549 cell line transfected with a non-targeting control (si-NTC) and a siRNA specific to KLF6-SV1 (si-SV1) demonstrate significant downregulation of KLF6-SV1 at both the mRNA and protein level at 72hr (*** $p < 0.001$). b, FACS analysis of siNTC and siSV1 transfected A549 cells at 72hr treated with cisplatin. Targeted reduction of KLF6-SV1 results in increased spontaneous apoptosis in the A549-CR cell line alone and in combination with 20 μm cisplatin (*** $p < 0.001$). c, qRT-PCR analysis reveals a marked upregulation of NOXA expression both with si-SV1 alone and in combination with cisplatin (** $p < 0.01$; *** $p < 0.001$). All experiments were repeated three independent times and data represents NOXA expression normalized to both GAPDH and B-actin levels. d, Western blot analysis demonstrates a marked upregulation of Caspase 3 and 8 and NOXA in siSV1 transfected chemoresistant A549 cells (A549-CR).

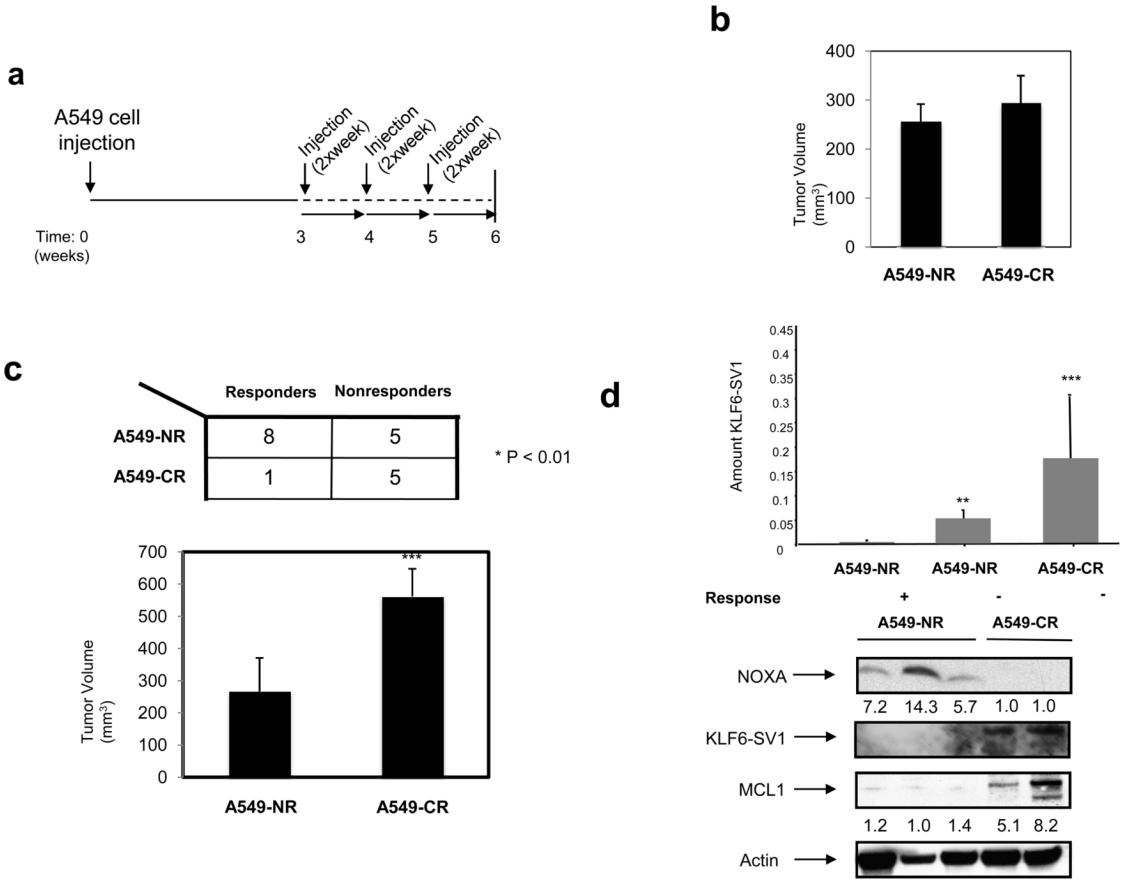

Figure 3.

The A549-CR cell line is resistant to cisplatin treatment in vivo and express increased levels of KLF6-SV1. a, Treatment protocol for the A549-CR and parental A549-NR cell lines. b, Starting tumor volumes in the two groups are equal. c, Treatment of the parental A549 derived tumors (A549-NR) with cisplatin resulted in a response rate 64% compared to 16% in the chemoresistant A549-CR cell line (* $p < 0.01$). The A549-CR tumors were on average 40% larger than the A549-NR at the end of the 3 week treatment period (*** $p < 0.0001$) d, Cisplatin resistant tumors express higher levels of KLF6-SV1. Tumors were excised at the end of the treatment period and RNA and protein were isolated. qtRT-PCR of the A549-NR and A549-CR derived tumors for KLF6-SV1 revealed a significant increased in KLF6-SV1 expression in both relatively resistant A549-NR tumors that failed to respond to cisplatin treatment (** $p < 0.01$) and in the A549-CR derived tumors (*** $p < 0.001$). Cisplatin resistant tumors express higher levels of the anti-apoptotic KLF6-SV1 and Mcl-1 and reduced levels of the proapoptotic Noxa. Western blot analysis of the A549-NR and A549-CR derived tumors revealed increased expression of SV1 and MCL1 with concomitant downregulation in Noxa expression.

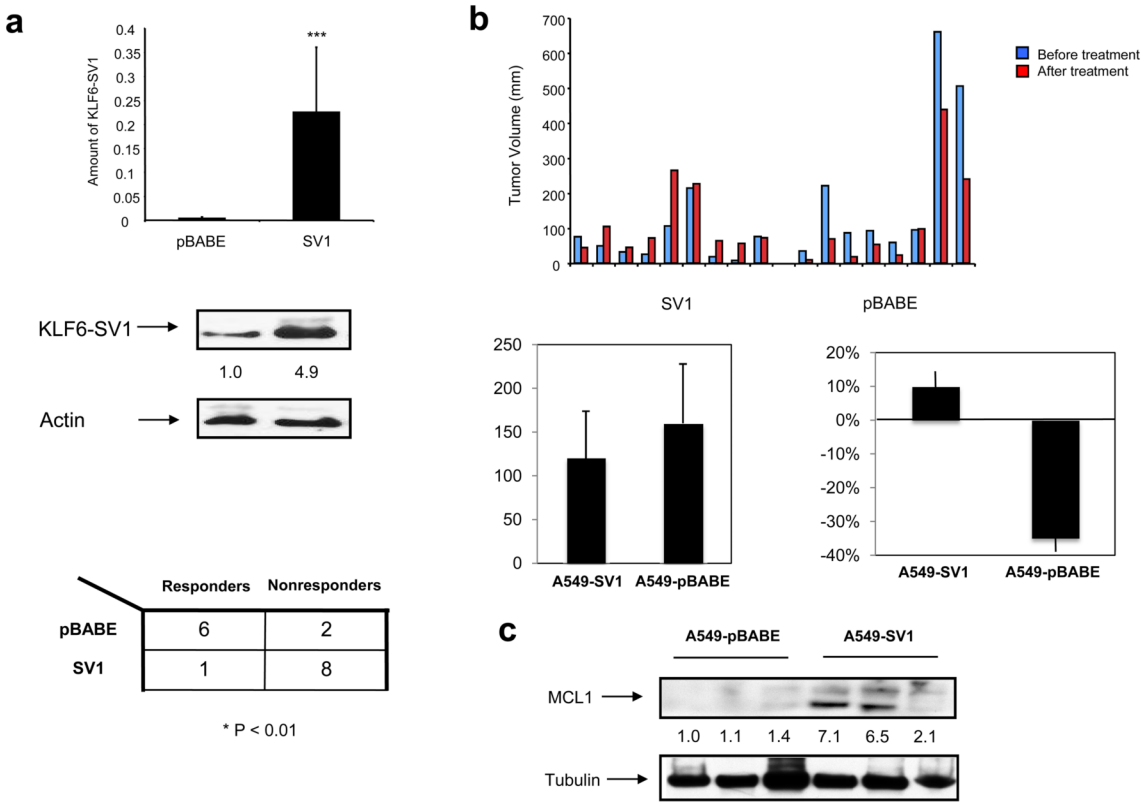

Figure 4.

Overexpression of KLF6-SV1 results in cisplatin resistance in vivo. a, qtRT-PCR analysis of pBABE and SV1 retrovirally infected A549 cells demonstrates a 10-fold overexpression of SV1 in pBABE-SV1 infected cell lines compared to control cells (pBABE) (*** $p < 0.001$). b, A549-SV1 derived tumors are resistant to cisplatin treatment. Treatment of the pBABE A549 control derived tumors with cisplatin resulted in a response rate 75% compared to 11% in the A549-SV1 overexpressing tumors (* $p < 0.01$). The bar graph represents tumor volume before and after treatment with cisplatin for each individual tumor in the pBABE and SV1 groups. There was no difference in the average starting volumes prior to treatment between the two groups. There was on average a 35% reduction in tumor volume in the A549-pBABE group over the 3 week treatment course compared to a 10% increase in tumor volume in the A549-SV1 derived tumors. c, Cisplatin treated A549-SV1 derived tumors express higher levels of the anti-apoptotic MCL1. Western blot analysis of protein derived from the A549-pBABE and A549-SV1 groups reveal a marked upregulation of Mcl-1 expression in SV1 expressing tumors compared to control.