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The *Klf6*-related super enhancer regulates *Klf6*-SV2 expression mediated proliferation in human hepatoma (HepG2) cells

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Abstract

Background: The *Klf6* gene, which belongs to Krüppel-like family of C2H2 zinc finger transcription factors, is greatly related to tumorigenesis via a high rate of somatic mutation in the carcinomas of prostate, liver, colon, stomach, lung, neck, pituitary, and nervous system: Furthermore, the pathways regulating the expressions of *Klf6* splice variants termed *Klf6-SV1*, *-SV2*, and *-SV3* remain obscure although their functional outcomes have been clear. In this study, the functional roles of *Klf6* variants in the inhibition of cell proliferation induced by the disruption of *Klf6*-related super enhancer in human hepatoma (HepG2) cells were evaluated.

Results: As a result, the disruption of *Klf6*-related super enhancer not only induced the upregulation of *Klf6-SV2* but also led to a significant reduction of proliferation in HepG2 cells. In addition, the disruption of *Klf6*-related super enhancer led to the induction of *p21* and *Bax* genes mediated by the upregulation of *Klf6-SV2*.

Conclusion: In conclusion, it was demonstrated that *Klf6*-related super enhancer modulates cell proliferation via the regulation of *Klf6-SV2* expression in human hepatoma (HepG2) cells. The results provide the functional significance of *Klf6*-related super enhancer in understanding the transcriptional regulation mechanism of *Klf6*.

Keywords: Super Enhancer, *Klf6* gene, Genome editing, CRISPR, *Klf6* isoforms

1 Background

Liver cancer is one of the most common six malignant tumors with high malignancy and a poor prognosis around the world. Although the genetic information and the risk factors of liver cancer formation have been increasingly identified, the exact mechanisms underlying the initiation, development, and metastasis of liver cancer are poorly understood [1].

The *Klf6* gene, which belongs to Krüppel-like family (containing C2H2 zinc finger transcription factors), is greatly related to tumorigenesis via a high rate of somatic mutation in the carcinomas of prostate, colon, stomach, liver, lung, neck, pituitary, nervous system, etc. [2, 3]. The functional role of dysregulated alternative splicing in cancers is now recognized in a range of human cancers including liver, lung, stomach, prostate, colon, etc. It has been reported that many genes associated with cancer can be alternatively spliced, and their expression can lead to the production of multiple splice variants with antagonistic function. Generally, the *Klf6* gene, which is known to be an important gene in the initiation, development, and metastasis of tumors, can be alternatively spliced into biologically active isoforms called *Klf6-SV1*, *-SV2*, and *-SV3* [4, 5]. In particular, the *SV1* variant has been shown to accelerate the progression and metastasis of prostate cancer [6, 7]. The functional role of *Klf6-SV1* in tumorigenesis was most well-defined among three *Klf6* spliced variants because it can be highly expressed in many human malignant tumors, and its over-expression is observed in cancer patients with poor prognosis and low survival rate [8–10]. Growing results have shown that elevated expression of *Klf-SV1* variant partially promotes the metastasis, progression, and mortality of cancer through its antagonism to *Klf6* [11]. Furthermore, elevated *Klf6-SV1/Klf6* mRNA ratios have been demonstrated with their functional characteristics in liver cancer samples. On the other hand,

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over-expression of *SV2* variants in IHH and HepG2 cells was observed to significantly reduce cell proliferation by apoptosis [12–14]. Although the functional outcome of *Klf6* splice variant has become clear, the pathway regulating expression of *Klf6* splice variant remains ambiguous.

Recently, a large cluster of transcriptional enhancers called super enhancers (SEs) have been identified. Furthermore, SEs have been shown to specifically regulate cell identity in many tumors, including liver, lung, stomach, and prostate, as genomic domains that are large non-coding enhancers [15–20]. We have already identified a *Klf6*-related super enhancer in human HepG2 cells using genomic editing techniques and demonstrated that *Klf6*-related SE regulates cellular proliferation as a potent regulator of *Klf6* gene expression [21]. We assumed that regulation of cell proliferation by *Klf6*-related super enhancer may be associated with *Klf6* variants in deeper study.

In this study, we obtained the cell clones of a *Klf6*-related super enhancer deleted by means of the CRISPR/Cas9 system-mediated genetic engineering as previously described [21] and also found that inhibition of cell proliferation by deletion of *Klf6*-related super enhancer is caused by *Klf6* variants in human hepatoma (HepG2) cells. At first, it was demonstrated that deletion of *Klf6*-related super enhancer induces the upregulation of *SV2* variant and leads to the inhibition of cell proliferation in HepG2 cells. In addition, it was shown that *Klf6*-related super enhancer leads to the induction of *p21* (cell-cycle-controlling gene) and *Bax* (pro-apoptotic gene) genes mediated by upregulation of SV2 variant. The results provide the functional significance of *Klf6*-related super enhancer in understanding the transcriptional regulation mechanism of *Klf6*.

2 Methods and materials

2.1 Cell culture and transfection experiments

HepG2 was derived from the Chinese Academy of Science. The cell culture and transfection were carried out according to cell culture and transformation protocols as previously described [21].

2.2 RNA extraction, reverse transcription, and real-time PCR

Total RNA isolation was performed using TRIzol (Life Tech) extraction for the target clones.

A total of RNA (1 μg) was reverse transcribed using the first-strand complementary DNA synthesis with random primers. RT-PCR was performed with *KLF6* 5′ and 3′ untranslated region-specific primers on 10 ng cDNA derived from the target clones as previously described [9]. qRT-PCR was carried out with SYBR Green PCR Master Mix (Applied Biosystems). The relative expression ratio of genes in the cells was quantified by the $2^{-\Delta\Delta CT}$ method.

Primers selected for the experiments are as following: 5′-CGGACGCACACAGGAGAAAA-3′/5′-CGGTGTGCTTTCGGAAGTG-3′ (*Klf6*), 5′-CCTCGCCAGGGAAGGAGAA-3′/5′-CGGTGTGCTTTCGGAAGTG-3′ (*Klf6-SV1*), 5′-TCGGGGAAGCCAGGAGAA-3′/5′-CGGTGTGCTTTCGGAAGTG-3′ (*Klf6-SV2*), 5′-CGGACGCACACAGGTGTT-3′/5′-TCTGCTCCCTCAGAGGTGCC-3′ (*Klf6-SV3*), 5′-ACTCTCAGGGTCGAAAACGG-3′/5′-CCTCGCGCTTCCAGGACTG-3′ (*P21*), 5′-ATCCAGGATCGAGCAGGGCG-3′/5′-ACTCGCTCAGCTTCTTGGTG-3′ (*Bax*), 5′-GTGTGGCGACATATGCAGCT-3′/5′-CAAGATCAGCAGTCTCATTC-3′ (*beta-actin*).

The analysis proceeded in triplicate, and the relative level of gene expression was assessed as *beta-actin*.

2.3 Cell proliferation assay and Western blot assay

Proliferation was determined by estimating [3H] thymidine incorporation as previously described [9]. Separation of proteins for Western blot assays proceeded on 10–15% SDS-PAGE gel as previously described [21]. The *beta-actin* protein was used as a loading control.

2.4 Plasmid construction and transfection

The pSUPER-siSV1, -siSV2, and -siSV3 plasmids used to downregulate *Klf6-SV1, -SV2* and *-VS3* expression were constructed according to the previously described method, and the pSUPER-Luc construct was used as a control [9]. The pSUPER-si-wt*KLF6* construct was generated as previously described using the pSUPER vector [22]. To insert the targeting sequence, DNA oligonucleotides were designed and cloned into the *Bgl*II-*Hind*III sites of the pSUPER vector. The characteristics of the designed DNA oligonucleotides are shown in Table 1.

Generation of cell clones over-expressing *Klf6-SV* variants was performed as previously described [12]. The coding sequences of *Klf6* and *Klf6-SV* variants were obtained by PCR amplification and inserted into p3XFLAG-CMV-10 vector (Sigma).

3 Results

3.1 Deletion of Klf6-related SE induces inhibition of cell proliferation in human HepG2 cells

A clone of *Klf6*-related SE deletion (SE-del) clone was obtained using the CRISPR/Cas9 system to evaluate the effect of *Klf6*-related SE on the expression of *Klf6* gene in HepG2 cells. The experimental procedure for obtaining SE-del clones in HepG2 cells has been detailed in previously published literature [21]. Expression of *Klf6* gene in SE-del clone using

Table 1 DNA oligonucleotides for pSUPER vector

Klf6 siSV1-*F*	GATCCCCAGGCTTTTCTCCTTCCCTGGC ttcaagagaGCCAGGGAAGGAGAAAAGCCTTTTGGAAA	*Klf6* siSV1-*R*	AGCTTTTCCAAAAAGGCTTTTCTCCTTCCCTG GCtctcttgaaGCCAGGGAAGGAGAAAAGCCTGGG
Klf6 siSV2-F	GATCCCCGCCAGGAGAAAAGCCTT ACttcaagagaGTAAGGCTTTTCTCCTGGCTTTTTGGAAA	*Klf6* siSV2-R	AGCTTTTCCAAAAAGCCAGGAGAAAAGCCTTA CtctcttgaaGTAAGGCTTTTCTCCTGGCGGG
Klf6 siSV3-F	GATCCCCGCAAGCAGACGAGCCTC ACttcaagagaGTAAGGCGTATCTCGTGGCTTTTTGGAAA	*Klf6* siSV3-R	AGCTTTTCCAAAAAGCGAAGAGACAGGCCTTA CtctcttgaaGCCAGACTTTTCTCCTGGCGGG
Luc-F	GATCCCCTACTTCGAAATGTCCGT TCttcaagagaGAACGGACATTTCGAAGTATTTTTGGAAA	Luc-R	AGCTTTTCCAAAAATACTTCGAAATGTCCGTT CtctcttgaaGAACGGACATTTCGAAGTAGGG
Si-wt*KLF6*-F	GATCCCCTGGCGATGCCTCCCCCGACttcaagaga GTCGGGGGGAGGCATCGCCATTTTTGGAAA	Si-wt*KLF6*-R	AGCTTTTCCAAAAATGGCGATGCCTCCCCCGAC tctcttgaaGTCGGGGGAGGCATCGCCAGGG

qRT-PCR was decreased by 80% or more, and strong inhibition of *Klf6* gene expression was observed as shown in Fig. 1a.

Reduced expression of *Klf6* gene in SE-del clone was confirmed at the protein level by using Western blot, and expression of *Klf6* protein was also significantly inhibited in SE-del clone as compared with the control (Fig. 1b). After deletion of *Klf6*-related SE, the effect of cell proliferation was evaluated using [3H] thymidine incorporation assay, concurrent with the result of decreased *Klf6* expression, it observed a markedly inhibited proliferation (Fig. 1c).

3.2 Deletion of Klf6-related SE regulates expression of Klf6-SV variants in human HepG2 cells

As shown in the above results, since significant inhibition of cell proliferation as well as expression of *Klf6* after deletion of *Klf6*-related SE was observed, therefore, it attempted to analyze the expression characteristics of *Klf6* variants by RT-PCR.

The SE-del clone expression pattern through RT-PCR of cDNA derived from the target clone containing the SE-del clone, and the control clone revealed a strong inhibition of the full-length tumor suppressor *Klf6* concurrently with a severe change in the

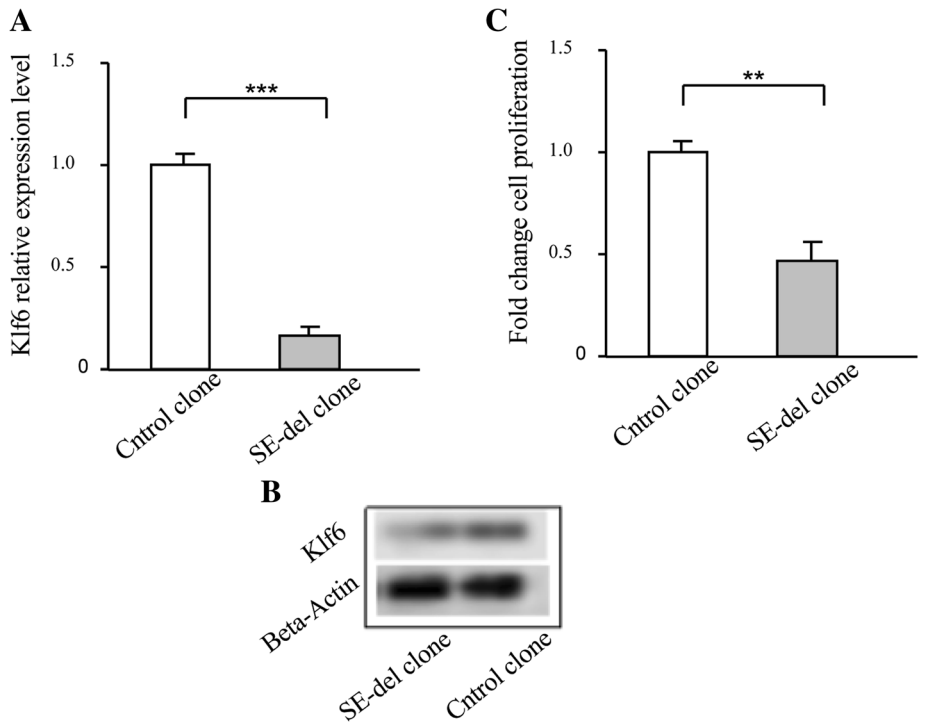

Fig. 1 *Klf6* gene expression and cell proliferation assay. **a** *Klf6* gene expression assay. The *Klf6* expression level was measured by qRT-PCR. The results represent the mean of five amplifications, ***$p < 0.001$. **b** *Klf6* proteins expression assay. *Klf6* proteins were determined by western blot. **c** Cell proliferation assay. [3H] thymidine incorporation was determined at 72 h. The results represent the mean of eight experiments, **$p < 0.01$

relative expression level of the *Klf6* splice variants *Klf6-SV1*, *-SV2*, and *-SV3* (Fig. 2a). Quantitative real-time PCR (qRT-PCR) analysis of cDNA derived from the target clones showed that the *SV/Klf6* mRNA ratio was increased in *Klf6-SV1* and *Klf6-SV2*, but decreased in *Klf6-SV3*. Especially, the expression of *Klf6-SV2* was three times higher than that of the control, but the expression of *Klf6-SV1* was increased very little. These results show that expression of *Klf6* splice variants in human HepG2 cells is strongly regulated by *Klf6*-related super enhancer, and in particular, inhibition of cell proliferation after deletion of SE is likely to be associated with over-expression of *Klf6-SV2*.

3.3 Deletion of Klf6-related SE induces inhibition of proliferation through over-expression of Klf6-SV2 in human HepG2 cells

On the basis of the above findings, we conducted an experiment to determine whether over-expression of *Klf6-SV2* induced by the disruption of the *Klf6*-related SE is actually associated with cell proliferation in human HepG2 cells. Firstly, we specifically inhibited the expression of *Klf6-SV1* and *-SV2*, which showed increased expression after the disruption of the *Klf6*-related SE using target-specific siRNAs in SE-del clone (Fig. 3). Targeted reduction of *Klf6-SV1* and *-SV2* expression in SE-del clone was confirmed at the mRNA level by qRT-PCR (Fig. 3a). Comparing to the siLuc control cells, the expression level of *Klf6*-SV1 in the siSV1 cells was decreased by at least 45% ($p < 0.01$), whereas the expression level of *Klf6*-SV2 in the siSV2 cells was decreased by at least 55% ($p < 0.05$). The effects of *Klf6-SV1* and *-SV2* inhibition were determined by measuring cell proliferation (Fig. 3b). Cell proliferation was increased twofold in siSV2 cells ($p < 0.01$) but decreased by half in siSV1 cells ($p < 0.01$). The results indicate that over-expression of *Klf6-SV1* promotes cell proliferation, but its downregulation induces inhibition. On the other hand, over-expression of *Klf6*-SV2 inhibits cell proliferation, but its down-expression promotes cell proliferation and thus appears to be responsible for inhibition of cell proliferation induced by deletion of the *Klf6*-related SE.

Secondly, we inhibited the expression of *Klf6-SV1*, *-SV2*, *-SV3*, and *wtKlf6* using target-specific siRNAs in SE-non-del clone (Fig. 3). As shown in Fig. 3c, d, down-regulation of *Klf6-SV1* (35%, $p < 0.05$) induces inhibition of cell proliferation, but down- regulation of *Klf6-SV2* (35%, $p < 0.05$) promotes cell proliferation. Expression of *Klf6-SV3* in siSV3 cells was decreased by at least 72% ($p < 0.05$) compared to the siLuc control cells, but there was no significant difference in cell proliferation analysis ($^{\#}p > 0.05$). However, expression of the wt*Klf6* in si-wt*Klf6* cells was decreased by 55% ($p < 0.01$) but a significant increases in cell proliferation ($p < 0.01$). As shown above, the results show that over-expression of *Klf6-SV2* induced by the disruption of the *Klf6*-related SE is an important factor associated with cell proliferation in human HepG2 cells, especially inhibition of cell proliferation.

3.4 Over-expression of Klf6-SV2 induces an increased expression of p21 and Bax in human HepG2 cells

The expression of cell cycle-regulated genes were analyzed to elucidate the proliferation-related mechanism induced by *Klf6-SV2*. In response to over-expression of *Klf6-SV2*, cell cycle-regulating *p21* (*CIP/WAF1*) expression was higher about two-fold in SE-del clone than that of control clone (Fig. 4a). Elevated expression in *p21* (*CIP / WAF1*) protein was also identified

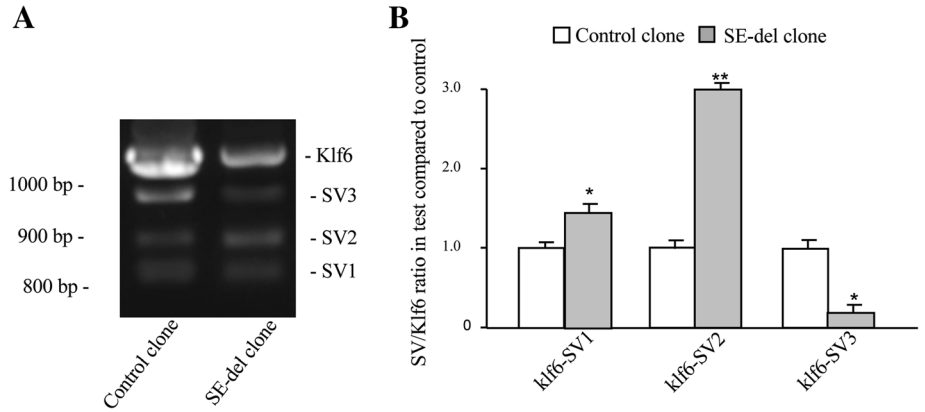

Fig. 2 Expression of *Klf6-SV* isoforms in the target clones. **a** RT-PCR of cDNAs with *Klf6* variant-specific primers [9]. PCR products were visualized after agarose gel electrophoresis and ethidium bromide staining. **b** *SV/Klf6* mRNA ratio analysis by qRT-PCR. The figure shows *Klf6-SV* expression relatively to *Klf6* expression in the target clones. The results are the mean of three different quantifications, $^{*}p < 0.05$, $^{**}p < 0.01$

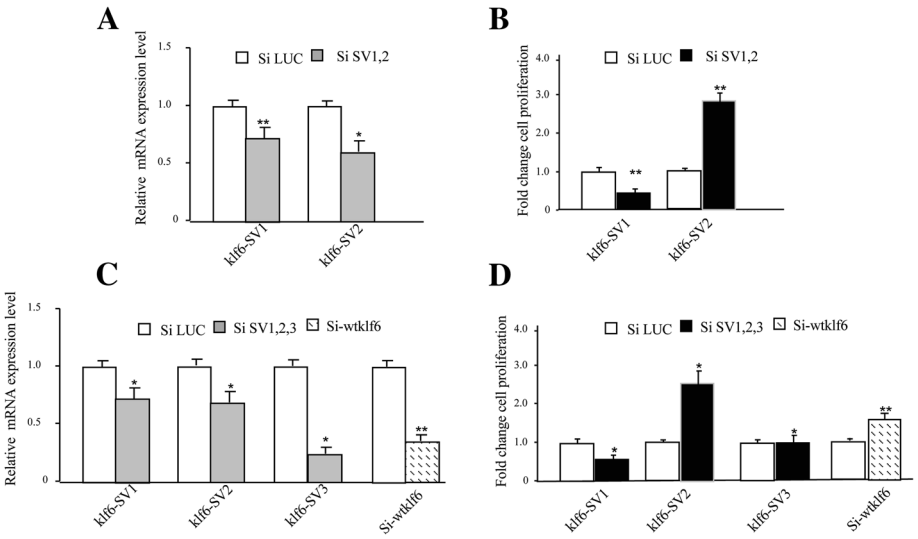

Fig. 3 Expression of *Klf6-SV* isoforms and cell proliferation assay in the target clones. **a** Expression assay of *Klf6-SV1* and *-SV2*. The SE-del clone cells were transfected with pSuper-siSV1, 2 or control pSuper-siLuc. **b** Cell proliferation assay. [3H] thymidine incorporation was determined at 72 h. Each experiment was performed in triplicate, $^{**}p < 0.01$. **c** Expression assay of *Klf6-SV* isoforms*:* The SE-non-del clone cells were transfected with pSuper-siSV1,2,3 or control pSuper-siLuc. The SE-non-del clone cells were transfected with pSuper-si-wt*Klf6* or control pSuper-siLuc. The results represent the mean of three amplifications, $^{**}p < 0.01$,$^{*}p < 0.05$. **d** Cell proliferation assay. Each experiment was performed in triplicate, $^{**}p < 0.01$,$^{*}p < 0.05$,$^{\#}p > 0.05$

by Western blot (Fig. 4b). A similar method was used to elucidate the apoptotic mechanism induced by over-expression of *Klf6-SV2*. Among the proteins involved in apoptosis, elevated expression of the pro-apoptotic *Bcl-2*-associated *Bax* gene was demonstrated (Fig. 4c). Increased expression in *Bax* was also confirmed by Western blotting (Fig. 4b), and the results suggested that *Klf6-SV2*-induced apoptosis is associated with the mitochondrial apoptotic pathway. Knockdown of *Klf6-SV2* expression by siRNA (Fig. 4a (+), c (+)) induces inhibition of *p21* and *Bax* at the protein (Fig. 4b) and mRNA (Fig. 4a, c). As a result, *Klf6-SV2* exhibits partially mediated anti-proliferative and pro-apoptotic effects through induction of *p21* (*CIP / WAF1*) and *Bax*.

Knockdown of *Klf6* variants by various *siRNAs* in SE-non-del clone can be observed in which over-expression of *Klf6-SV2* increases the expression of *p21* and *Bax* at the protein level (Fig. 5c) and mRNA (Fig. 5a, b). In addition, over-expression of various variants in SE-non-del clone also demonstrated that increased expression of *p21* and *Bax* are dependent on over-expression of *Klf6-SV2* (Fig. 5d–f]. As shown in Fig. 5, unlike the other variants, *Klf6-SV3* did not affect the expression of *P21* and *Bax* genes ($^{\#}p > 0.05$). Upregulation of *Klf6-SV1* reduces the expression of *P21* and *Bax* genes, but its

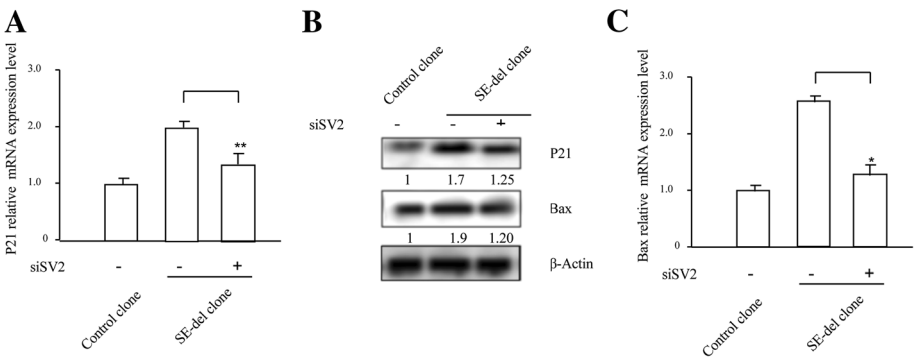

Fig. 4 Over-expression of *Klf6-SV2* triggers increased expression of *p21* and *Bax*. **a** Expression of *p21* mRNA in SE-del clone transfected with siSV2. The results represent the mean of three amplifications, $^{**}p < 0.01$. **b** Expression of *p21* and *Bax* proteins in SE-del clone transfected with siSV2. The gels are representative of three analyses. **c** Expression of *Bax* mRNA in SE-del clone transfected with siSV2. The results represent the mean of three amplifications, $^{*}p < 0.05$

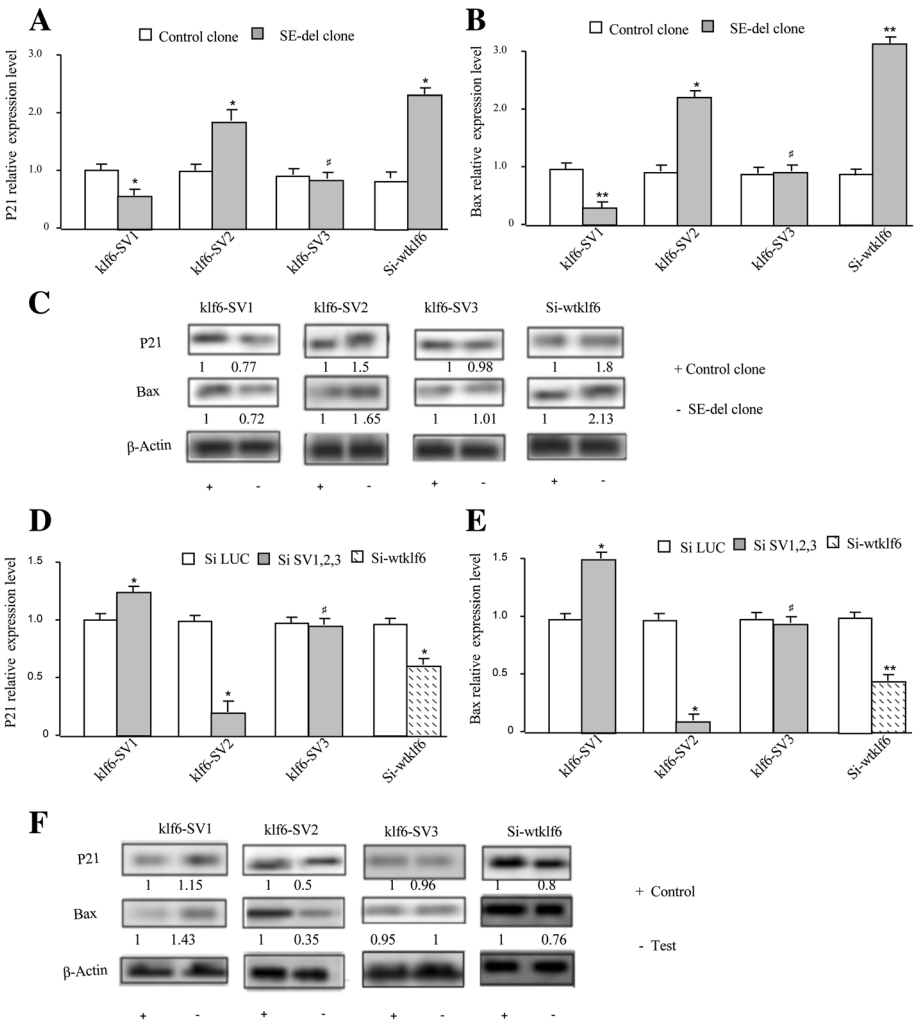

Fig. 5 The effect of *Klf6* variants on the expression of *P21* and *Bax* genes. **a** Expression of *p21* mRNA in SE-non-del clone transfected with various siRNA. The results represent the mean of three amplifications, $^{\#}p > 0.05$, $^{*}p < 0.05$. **b** Expression of *Bax* mRNA in SE-non-del clone transfected with various siRNA. The results represent the mean of three amplifications, $^{**}p < 0.01$, $^{*}p < 0.05$,$^{\#}p > 0.05$. **c** Expression of *p21* and *Bax* proteins in SE-non-del clone transfected with various siRNA. **d** Expression of *p21* mRNA in SE-non-del clone with over-expression of various *Klf6* variants. The results represent the mean of three amplifications, $^{\#}p > 0.05$, $^{*}p < 0.05$. **e** Expression of *Bax* mRNA in SE-non-del clone with over-expression of various *Klf6* variants. The results represent the mean of three amplifications, $^{**}p < 0.01$, $^{*}p < 0.05$,$^{\#}p > 0.05$. **f** Expression of *p21* and *Bax* proteins in SE-non-del clone with over-expression of various *Klf6* variants

downregulation increases expression of *P21* and *Bax* genes.

The results show that *Klf6*-related super enhancer leads to an induction of the cell cycle-regulating *p21* and the pro-apoptotic *Bax* genes mediated by up-regulation of *SV2* variant.

4 Discussion

The *Klf6* transcription factor is closely related to tumorigenesis through high somatic mutation rates in multiple carcinomas including prostate, colon, stomach, liver, lung, and neck [2, 3]. The role of dysregulated alternative splicing in cancer is now being studied in a variety of cancers, and it is confirmed that many cancer-related genes are alternatively spliced and their expression leads to the production of multi-splice variants with antagonistic function. *Klf6* is alternatively spliced into biologically active isoform called *Klf6-SV1*, *-SV2*, and *-SV3* [4, 5]. Although the functional outcomes of *Klf6* splice variants have become clearer, the pathways regulating expression of *Klf6* splice variants remain obscure. Therefore, the observation of expression levels of *Klf6* variants and their respective functions in human HepG2 cells is essential for further understanding of their mechanisms. Disruption of *Klf6*-related SE induces inhibition of proliferation in human HepG2 cells. We hypothesized that inhibition of cell proliferation

induced by the disruption of *Klf6*-related super enhancer may be associated with *Klf6* variants. In our study, the results show that *Klf6*-related super enhancer regulates expression of *Klf6* variants in human HepG2 cells. As shown in Fig. 2b, the expression of *Klf6-SV2* was three times higher than one of the control, but the expression of *Klf6-SV1* was increased very little. Specifically, the functional modeling of findings using siRNA specific to *Klf6-SV2* in human HepG2 cells revealed that changes in expression level directly affect cell proliferation. We conducted an experiment to determine whether the over-expression of *Klf6-SV2* induced by disruption of *Klf6*-related SE is actually associated with cell proliferation in human HepG2 cells. As shown in Fig. 3, down-expression of *Klf6-SV*1, *-SV*2, and *-SV*3 inhibited cell proliferation in siSV1 cells and promoted in siSV2 cells, but not in siSV3 cells. Furthermore, cell proliferation was shown to be directly associated with *Klf6-SV2* levels. These results show that over-expression of *Klf6-SV2* induced by disruption of *Klf6*-related SE is an important factor associated with inhibition of cell proliferation in human HepG2 cells. The above results are further supported by literature [1, 7, 9, 12, 13, 23] that over-expression of *Klf6-SV1* promotes cell proliferation, while over-expression of *Klf6-SV2* inhibits cell proliferation. It is also consistent with data that downregulation of wt*Klf6* promotes cell proliferation in hepatocellular carcinoma (HCC) [24]. We demonstrated that over-expression of *Klf6-SV2* induces transcriptional activation of *p21 (CIP/WAF1)* and *Bax* genes (Fig. 5). The results show that over-expression of *Klf6-SV2* exerts partially mediated anti-proliferative and pro-apoptotic effect by induction of *p21* (*CIP / WAF1*) and *Bax*. The results are consistent with the literature [1, 9, 12, 13, 23] showing that over-expression of *Klf6-SV2* induces anti-proliferation and apoptosis.

We proposed a novel model regulating cell proliferation via modulation of *Klf6-SV2* expression into the growth-relating isoforms (Fig. 6). *Klf6*-related super enhancer regulates expression of *Klf6-SV2*, thereby altering the relative ratio of *Klf6* to *Klf6-SV2*, which leads to inhibition of cell proliferation. This model requires further studies, such as what is the signaling pathway between the super enhancer and biologically active isoforms, and how it is regulated [25–29].

5 Conclusions

Several studies have identified new cancer therapeutics that target super-enhancers of various tumor types. In our study, we have elucidated the functional properties of the *Klf6*-related super enhancer on the expression of *Klf6* splice variants which may be helpful in the target treatment of *Klf6*-related super enhancer. the disruption of *Klf6*-related super enhancer showed not only induced the up-regulation of *Klf6-SV2* but also led to a significant reduction of proliferation in HepG2 cells. It was demonstrated that the disruption of *Klf6*-related super enhancer led to the induction of *p21* and *Bax* genes mediated by the up-regulation of *Klf6-SV2*. Therefore, it showed that Klf6-related super enhancer modulates cell proliferation via the regulation of *Klf6-SV2* expression in human hepatoma (HepG2) cells. The results provide the functional significance of *Klf6*-related super enhancer in understanding the transcriptional regulation mechanism of Klf6.

But this result raises an interesting question of how cellular signals converge to regulate splicing events in promoting tumor progression. Targeted analysis of molecular pathways of *Klf6*-related super enhancer that regulates *Klf6-SV2* expression will provide further insight into the role of this gene in the progression of human cancer.

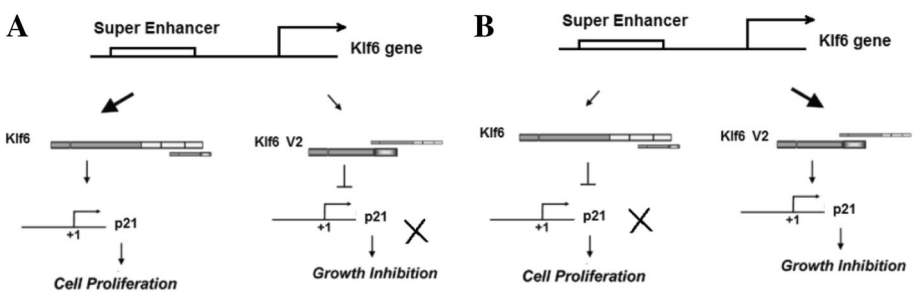

Fig. 6 The model regulating cell proliferation by super enhancer. **a** Pre-disruption model of *Klf6*-related SE. When *Klf6*-related SE is not disrupted, it upregulates *Klf6* expression and induces cellular proliferation. **b** Post-disruption model of *Klf6*-related SE. When *Klf6*-related SE is disrupted, it upregulates *Klf6-SV2* expression which leads to *p21*-mediated growth inhibition. The size of the arrow in the figure represents the relative expression intensity of the target gene. In other words, an arrow indicated by a thick line means strong gene expression compared to a thinly marked arrow

Abbreviations
Klf6: Kruppel-like factor 6; wtKlf6: Wild-type Klf6; qRT–PCR: Quantitative real-time PCR; WT: Wild type; SEs: Super enhancers; SE-del clones: SEs deleted clone; SE-non-del clone: SE-non-deleted clone; siRNA: Small interfering RNA; Luc construct: Luciferase constructs

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Authors' contributions
RKC was responsible for the overall project design and experiments, and KC was involved in the transfection experiments of HepG2. CSI was engaged in cell proliferation assays and Western blot experiments. SJH and OSH performed Klf6-SV1, -SV2, and -VS3 expression experiments. All authors have read and approved the manuscript.

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Competing interests
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