Metformin Affects Cancer Regulation and Metabolism of Oral Squamous Cell Carcinoma Partially Through Upregulation of H3K27ac

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Primary research

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Abstract

Objectives

Metformin, a first-line drug that has been used for type 2 diabetes treatment, recently attracts broad attention for its therapeutic effects on diverse human cancers. However, its effect and underlying mechanisms in oral squamous cell carcinoma (OSCC) are not well known.

Materials and Methods

OSCC cells were used to detect the effect of metformin on cell proliferation, colony formation, cell cycle and migration in vitro. Tumor growth of nude mice was conducted to detect the effect of metformin in vivo. Western blotting and immunohistochemistry were used to investigate the effect of metformin on the expression of histone modification in vitro and vivo. The combined effect on cell proliferation and histone modification of metformin and downregulation of EZH2 was detected by CCK8 and western blotting. Additionally, RNA-seq and ChIP-seq was performed to explore the underlying mechanisms of metformin in OSCC.

Results

Metformin could inhibit OSCC cell proliferation and tumor growth with the increased acetylation at lysine 27 of histone H3 (H3K27ac) in vitro and vivo. The underlying mechanisms were related to cancer regulation and cancer metabolism, affected by the increased H3K27ac. Additionally, metformin could synergize with siRNA-EZH2 to inhibit OSCC cell proliferation independent on the increased H3K27ac.

Conclusions

Metformin could play anti-cancer role in OSCC progression, with the reprogramming of cancer regulation and metabolism partially regulated by the increased H3K27ac.

Introduction

Oral cancer, with rising incidence in all age groups, has been a growing problem globally and a top cause of mortality in some regions[1, 2]. Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer[1, 2]. Multidisciplinary approach has been advocated for oral cancer treatment with the purpose of improving outcomes. Neoadjuvant chemotherapy and palliative chemotherapy are potential and effective to improve outcomes[3]. Moreover, there is a potential role of the combination of traditional therapies and immunotherapy in oral cancer. However, all the current therapies are associated with some obvious adverse effects [4], [5, 6]. Thus, it will be beneficial to apply a safe chemotherapy method with rare side effects.

Metformin, which is safe and off patent, has been turned out to be a promising anti-cancer drug for many malignancies in clinical studies[7]. It has been reported that metformin might reduce oral cancer risk in

diabetics[8]. More interesting, metformin synergizes with traditional therapies and rescues the therapeutic resistance[9-13]. The canonical anti-cancer mechanism of metformin is that it causes energy starvation with activated AMPK and inhibited mTOR *in vitro* and *in vivo*[7]. In addition, histone modification attributes to the anti-cancer effect of metformin by regulating gene expression[14, 15]. Metformin causes an indirect inhibition on histone deacetylases (HDAC) and decreases the level of tri-methylation at lysine 27 of histone H3 (H3K27me3) by disrupting the catalytic activity of the polycomb repressive complex 2 (PRC2)[14, 16]. The decreased H3K27me3 is concurrent with the upregulated acetylation at lysine 27 of histone H3 (H3K27ac), which results in activated transcription of genome[17]. It remains less exploration on the association between anti-cancer effect of metformin and global histone modification. Hence, our study aimed to reveal the potential role and mechanism of metformin in regulating the anti-cancer effect via global modification level of H3K27me3 and H3K27ac.

In this study, we showed the anti-cancer effect of metformin on OSCC with the globally increased H3K27ac level *in vitro* and *in vivo*. The linked mechanism has been investigated by RNA-seq and ChIP-seq. We found that metformin regulated the carcinogenesis and metabolism of OSCC partially through pathways associated with H3K27ac modification.

Materials And Methods

Cell culture and chemicals

Cal27 and HSC-2 were obtained from State Key Laboratory of Oral Diseases, Sichuan University, China. Cells were cultured in high glucose DMEM (Gibco, USA), supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin and streptomycin (100 μg/ml) (Hyclone, USA). Metformin was purchased from Sigma-Aldrich (USA).

Cell proliferation assay

Cal27 and HSC-2 cells (3×10^3) were seeded into 96-well plates and treated with 0, 2.5, 5, 10, 20 and 40 mM metformin for 24, 48, 72 and 96 h. The cells were incubated with Cell counting kit-8 (CCK8; Donjido, Japan) at 37 ℃ for 1.5 h following the manufacturer's instruction. The absorbance at 450 nm was detected by a microplate reader (Thermo, USA).

Colony formation

Cells (500 cells per well) were seeded into 6-well plates and incubated overnight allowing to cell adherence. Then cells were treated with 0, 5 and 10mM metformin for 14 days until the visible colonies were generated. After the fixation by 4% paraformaldehyde, colonies were stained with crystal violet for 5 min. The colonies were photographed by camera and counted under the phase-contrast microscope.

Cell cycle assay

Cal27 and HSC-2 cells (3×10^5) were seeded into the 6-well plates and treated with 10, 20 and 40 mM metformin for 48 h. Then cells were fixed with 75% ethanol at 4 ℃ for 4 h and the cell cycle was detected by using PI kit (KGA512, Jikai, China) following the recommended instruction.

Wound healing assay

Cal27 and HSC-2 cells (8×10^5) were seeded into 6-well plates and wounded by pipette tips after incubation overnight. Then cells were cultured with serum-free medium for 24 h. The images were captured using phase-contrast microscopy at 0 h and 24 h.

Tumorigenesis

Cal27 cells (5×10^6) in logarithm stage were suspended in 200 μl phosphate-buffered saline (PBS) and injected into the right armpit of 6-week-old BALB/C nude mice. When the tumors were palpable one week later after the injection, mice were randomly divided into the group treated with metformin and the other without metformin. For the treated group, metformin (200 μg/ml) was added into the drink water in a light-protected bottle and the control group were supplied with normal drink water. The animal weight, blood glucose and tumor volume were detected until the tumor volume was up to 1,000 mm^3. The tumor volume was calculated according to the formula: volume = (major diameter^2×minor diameter) / 2. The mice were sacrificed after 3-week treatment and tissues were fixed with 4% paraformaldehyde for hematoxylin-eosin (HE) stain and immunohistochemistry (IHC) analysis.

Immunohistochemistry

The IHC analysis was conducted according to the instruction of antibodies' manufacturers. The primary antibodies were as following: H3K27me3 (#9733, Cell Signaling Technology, USA) and H3K27ac (#8173, Cell Signaling Technology, USA).

OSCC samples collection

The OSCC samples and the corresponding adjacent normal tissues were obtained from the inpatients at West China School of Stomatology, Sichuan University. There were 2 patients with squamous cell carcinoma (SCC) on tongue, 2 on oral floor, and 1 on buccal. All patients were diagnosed as primary SCC and conducted with radical surgical resection. Informed consents of all patients were obtained.

Western blotting

Cells and tissues were lysed in RIPA (P0013B, Beyotime, China) supplemented with 1mM PMSF (ST506, Beyotime, China) and 0.01mM phosphatase inhibitors (P1260, Solarbio, China). Samples with 20 mg protein in each group was separated by 10% SDS-PAGE and electrotransferred to PVDF membranes (Millipore, USA). The primary antibodies were used as following: β-actin (1:2000, 20536-1-AP, Protein tech, China), EZH2 (1:1000, #5246, Cell Signaling Technology, USA), SUZ12 (1:1000, #3737, Cell signaling Technology, USA), EED (1:1000, 16818-1-AP, Protein tech, China), Histone 3 (1:2000, 17168-1-AP Protein

tech, China), H3K27me3 (1:1000, #9733, Cell Signaling Technology, USA), and H3K27ac (1:1000, #8173, Cell Signaling Technology, USA). After incubated with horseradish peroxidase-conjugated secondary antibody (1:2000, #7074, Cell Signaling Technology, USA), the blots were visualized with chemiluminescent substrate (US Everbright Inc., China) and quantified by Image Lab (Bio-Rad, USA).

siRNA transfection

Endofectin Max (Genecoopia) was used for siRNA transfection following the manufacturer's recommendation. Cells were transfected with the mixture of opti-MEM (Gibco, USA), endofectin and siRNA. Control siRNA was used as a negative group. After incubation for 48 h, cells were treated with 10mM metformin for 48 h. SiRNA was designed and purchased from Genepharma (China). The sequence of siRNA-EZH2 were GCAGCUUUCUGUUCAACUUTT, AAGUUGAACAGAAAGCUGCTT. The sequence of siRNA-Control was UUCUCCGAACGUGUCACGUTT, ACGUGACACGUUCGGAGAATT.

RNA-seq

OSCC cells, Cal27 and HSC-2, were treated with or without 10mM metformin for 6 days. According to the methods in previous study[18], cells (1×10^6) were lysed with Trizol reagent (Invitrogen) and oligo (dT) magnetic beads (Thermo, USA) were used to enrich mRNA. After mRNA was divided into small fragments, cDNA was synergized and purified with magnetic beads. The sequencing was carried out on Illumina Novaseq 6000 in Novogene Bioinformatics Technology (China). Control group consisted of Cal27 and HSC-2 treated without metformin and case group consisted of Cal27 and HSC-2 treated with 10 mM metformin. The data were analyzed by Novogene Bioinformatics Technology (China). Each group consisted of two replicates from Cal27 and HSC-2.

ChIP-seq

OSCC cells, Cal27 and HSC-2, were cultured with or without 10 mM metformin for 6 days. Then 1×10^8 cells were cross-linked using 1% paraformaldehyde for 5 min and quenched with 125mM glycine at room temperature. DNA was purified by associating with the antibodies of H3K27me3 (#9733, Cell Signaling Technology, USA), H3K27ac (#8173, Cell Signaling Technology, USA) and Normal Rabbit IgG (#2729, Cell Signaling Technology, USA) as previously described[19]. The DNA fragments were end-paired 5'-phosphorylated, 3'-dA-tailed and ligated to adapters using the Acegen DNA library Prep kit (Acegen, AG0810). The adapter-ligated DNAs were purified and amplified by 12 cycles of PCR using illumine 8-bp dual index primers. The constructed libraries were then analyzed by Agilent 2100 bioanalyzer and sequenced on Illumina Novaseq 6000 using a 150 × 2 paired-end sequencing protocol. The sequencing and data analysis were conducted in Novogene Bioinformatics Technology. Each group consisted of two replicates from Cal27 and HSC-2.

Statistics analysis

All data are presented as mean ± SD. All experiments were conducted three times independently except for extra interpretation. One-way analysis of variance (ANOVA) was used to compare the difference in multiple group and Student's t test was used to analyze the difference of two groups. $P<0.05$ was set to be statistical significance.

Results

Metformin attenuated cell proliferation, induced cell cycle arrest and inhibited cell migration of OSCC at high metformin concentration.

In order to elucidate the effect of metformin on OSCC cells, CCK8 and colony formation assays were performed to evaluate the cell proliferation, PI assay for cell cycle and wound healing assay for migration in vitro. The growth of OSCC cells treated with 10, 20 and 40 mM metformin was inhibited from the 3rd day ($p<0.05$, Fig. 1A, B). In line with the result of CCK8 assay, the colony formation of OSCC cells treated with metformin (5 and 10 mM) was significantly inhibited ($p<0.05$, Fig. 1C). And the cell cycle test results showed that cells treated with 20 and 40mM metformin was significantly arrested at G0/G1 stage ($p<0.05$, Fig. 1D). In addition, the migration of Cal27 and HSC-2 could be inhibited by 10mM metformin ($p<0.05$, Fig.1E).

Metformin inhibited tumorigenesis in animal model

The animal model was used to illustrate the anti-cancer effect of metformin on OSCC in vivo. The tumor volumes and mice weight were recorded every three days after the tumors were palpable. After three-week treatment, the mice were euthanized (Fig. 2A) and tumors were harvested and measured (Fig. 2B). At the end of the experiment, the tumor weight of mice treated with metformin was 20% less than that of control group ($p<0.05$, Fig. 2C). Consistent with the decreased tumor weight, the average tumor volume of mice treated with metformin was reduced by 39.28% compared with that of control group ($p<0.05$, Fig. 2D). Meanwhile, there was no significant side effect on animal weight and blood glucose in these two groups (data were not shown). Histological examination with HE stains showed there was no metastasis to the lungs and metformin had no associated toxicity on pancreas, liver, and kidney (Fig. 2E).

Metformin upregulated H3K27ac in OSCC cell lines and tumor tissues

To explore the effect of metformin on H3K27ac and H3K27me3 in OSCC, OSCC cells and tissues treated with or without metformin were used to detect the expression of H3K27ac and H3K27me3. As shown in Fig. 3A, Cal27 and HSC-2 treated with metformin showed higher expression of H3K27ac than that of control group ($p<0.05$). The expression of H3K27me3 in Cal27 and HSC-2 treated with metformin tended to be downregulated although there was no statistical significance ($p>0.05$, Fig. 3A). In vivo, the tumor samples of mice treated with metformin showed higher expression of H3K27ac and lower expression of H3K27me3 than in the control group (Fig. 3B). Meanwhile, our data showed that metformin had no effect on the expression of EZH2, SUZ12 and EED (Fig. 3A).

Metformin synergized with siRNA-EZH2to inhibit OSCC cell proliferation

Recent studies showed that EZH2 was a crucial enzymatic subunit on catalyzing the methylation of the H3K27[16]. To determine the role of increased H3K27ac and decreased H3K27me3 in OSCC, we performed siRNA to downregulate the expression of EZH2 in order to decrease the expression of H3K27me3 and increase the expression of H3K27ac. And we found that EZH2 was overexpressed in OSCC tissues (p<0.05, Fig. 4A). Then, CCK8 assay was used to detect the combined effect of siRNA-EZH2 and metformin on OSCC cell proliferation (Fig 4B). Our data showed that siRNA-EZH2 significantly inhibited OSCC cell growth, which could be enhanced by adding metformin from 48h (p<0.05, Fig. 4B). However, the combination of metformin and siRNA-EZH2 showed no significant effect on the increased level of H3K27ac and decreased level of H3K27me3. (p>0.05, Fig 4C).

The possible upstream regulators and related pathways in response to metformin in OSCC cells

To elucidate the potential mechanism of metformin on OSCC cells, we performed RNA-seq on Cal27 and HSC-2 cells treated with and without 10mM metformin for 6 days (Fig. 5A). In order to avoid batch effects, all samples were processed and sequenced at the same time. There were 147 overlapping differential genes (96 of up, 51 of down) in Cal27 and HSC-2 cells treated with metformin compared with the control (Fig. 5B). Most differential genes were associated with important biological processes in cancers. All differential genes were with a $\log_2$ fold change ratio cutoff>1 and p value<0.05 (Fig. 5B-D). Gene ontology (GO) analysis showed that these genes were mainly related to response to peptide, angiogenesis, response to nutrient levels, cellular amino acid metabolic process, cellular response to extracellular response, fat cell differentiation and other GO terms (Fig. 5E). The enrichment pathways analysis showed that metformin could cause a series of carcinogenesis pathways involved in signal transduction, signaling molecules and interaction, Endocrine system, amino acid metabolism, carbohydrate metabolism, mistranslation and mis-transcription. (Fig. 5F). ENCODE Transcription Factor targets found that there were three significant upstream transcription factors, GATA3, SREBF1 and POU2F2 (Fig. 5G).

Metformin correlated to cancer regulation and cancer metabolism via modification of H3K27ac

To elucidate the role of H3K27me3 and H3K27ac in response to metformin on OSCC cells, the ChIP-seq was performed (Fig. 5A). There were 141 differential peaks related to H3K27ac in Cal27 and HSC-2 treated with and without metformin, while there were no common differential genes correlated with the modification of H3K27me3 in these two cell lines. (Fig. 5H). GO analysis on molecular function based on ChIP-Seq data found that mRNA binding and catalytic activity and acting on RNA were strongly affected by increased H3K27ac (Fig. 5I). And the results in GO analysis on cellular component also showed that the increased H3K27ac functioned partly through mitochondrial matrix, nuclear speck and mitochondrial protein complex (Fig. 5I). The enrichment analysis in ChIP-seq differential peaks related genes showed that the increased H3K27ac was associated with spliceosome, sugar metabolism, nucleotide metabolism and aminoacyl-tRNA biosynthesis (Fig. 5J).

Discussion

In this study, metformin was proved to be an anti-cancer drug on OSCC in vivo and in vitro, which was in line with the previous studies [20-24]. Metformin has been regarded as one inhibitor for Mitochondrial Complex I, which could induce the imbalance of cellular energy status and nutrient metabolism[25]. Consistent with the previous study[26], the results in this study showed OSCC cells treated with metformin could reprogram the amnio acid metabolism and citrate acid cycle. And the increased H3K27ac induced by metformin could regulate the reprogramming of sugar metabolism and nucleotide metabolism. Totally, our data were consistent with the previous studies that metformin treatment could cause metabolic reprogramming. To our knowledge, this is the first study to illustrate that increased H3K27ac caused by metformin in OSCC was involved in the process of the reprogrammed cancer metabolism. These findings present an insight into the anti-cancer mechanism of metformin on cancer metabolism in OSCC.

Apart from the effect of metformin on cancer metabolism, our study showed that metformin could dysregulate the biological process of transcription and translation in OSCC. The results showed that aminoacyl tRNA biosynthesis was affected by metformin treatment both in RNA-seq and Chip-seq results. And the dysregulation of aminoacyl tRNA biosynthesis indicated the change of protein biosynthesis in translation level[27]. In addition, the result in RNA-seq indicated the transcriptional misregualtion in cancer and the data in Chip-seq indicated dysregulation in spliceosome, which were both related to gene transcription. Moreover, several signal transduction pathways and endocrine system were involved in the anti-cancer mechanism of metformin in OSCC. These results indicate a deeper insight on the complicated anti-cancer mechanism of metformin, which could help to elucidate the role of metformin in OSCC. While our data cannot fully interpret the metformin's anti-cancer activity, they suggest that metformin exerts a crucial anti-cancer effect via regulating transcription and translation and inhibiting downstream pathways.

Histone modifications have been demonstrated to be predictive factors for cancer prognosis. The high expression of H3K27me3 and H3K27ac was shown to be associated with aggressive liver cancer and low expression of H3K18ac and H3K27me3 was illustrated to be related with better prognosis of esophageal cancer, while lower level of H3K27me1 and H3K27me3 was demonstrated to be correlated with short free disease survival in renal cell carcinoma[28, 29]. Likewise, evidences show that histone modification patterns are associated with oral cancer progression and outcome. In the study of oral cancer induced by 4-Nitroquinoline-1-oxide and ethanol, global overexpression of H3K27ac and H3K27me3 was shown to be correlated with oral carcinogenesis[30]. Low level of H3K9ac could be used as a biomarker for poor prognosis in oral cancer[31]. And low level of H3K4ac and high level of H3K27me3 were correlated with the aggressive oral cancer[32]. In our study, we found that cells and tumor tissues treated with metformin showed lower expression tendency of H3K27me3 and higher expression level of H3K27ac, which suggests that the global level of H3K27me3 and H3K27ac could serve as predictive biomarker for the response to metformin in vitro and in vivo. However, future studies should be conducted.

EZH2 was demonstrated to be overexpressed in OSCC and correlated with patients' poor prognosis[33, 34]. The overexpressed EZH2 abrogated the effect of metformin by increasing the level of H3K27me3 in ovarian cancer[35]. In our study, we showed overexpressed protein level of EZH2 in OSCC tissues. Although there was no synergistic effect on H3K27me3 and H3K27ac induced by metformin and siRNA-EZH2, the antiproliferative effect of metformin on OSCC could be enhanced by siRNA-EZH2. The synergistic effect of metformin and siRNA-EZH2 suggested that the suppression of oncogenic function of metformin in OSCC was not fully dependent on the increased H3K27ac. It has been reported that metformin could increase the acetylation of histone and non-histone protein in prostate and ovarian cancer cells[36]. In this regard, our study is consistent with previous study and elucidates the role and underlying mechanism of increased H3K27ac in OSCC. Furthermore, the combination of metformin and inhibitors of methyltransferase was possible for OSCC therapy.

Conclusions

Taken together, we showed that metformin inhibited OSCC cell proliferation and induce OSCC cell cycle arrest at higher concentration in high glucose culture medium. The anti-cancer mechanism of metformin involves abnormal regulation of cancer and reprogramming of cancer metabolism, which is partly regulated by increased H3K27ac.

Abbreviations

OSCC: oral squamous cell carcinoma; H3K27ac: acetylation at lysine 27 of histone H3; HDAC: histone deacetylases; H3K27me3: tri-methylation at lysine 27 of histone H3; PRC2: polycomb repressive complex 2; FBS: fetal bovine serum; CCK8: Cell counting kit-8; PBS: phosphate-buffered saline; HE: hematoxylin-eosin; IHC: immunohistochemistry; GO: Gene ontology.

Declarations

Ethics approval and consent to participate

This current study was approved by the Medical Ethics Committee of West China Hospital of Stomatology, Sichuan University (WCHSIRB-D-2019-026). The animal experiment followed the institutional and national guide for the care and use of laboratory animals.

Consent for publication

Not applicable.

Availability of data and materials

RNA-seq and ChIP-seq data have been submitted to the database of NCBI (BioProject: PRJNA660568). Other data of this current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no potential conflicts of interest in the publication of this study.

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Authors' contributions

Shan Liu and Xiaoru Hou did the experiments and drafted the manuscript; Congyu Shi and performed the statistical analysis; Xiaoyi Wang, Chunjie Li and Xiangrui Ma critically reviewed the manuscript; Pan Gao and Xiaoyi Wang managed the experimental design, reviewed the manuscript. Pan Gao and Xiangrui Ma provided funding support.

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Figures

Figure 1

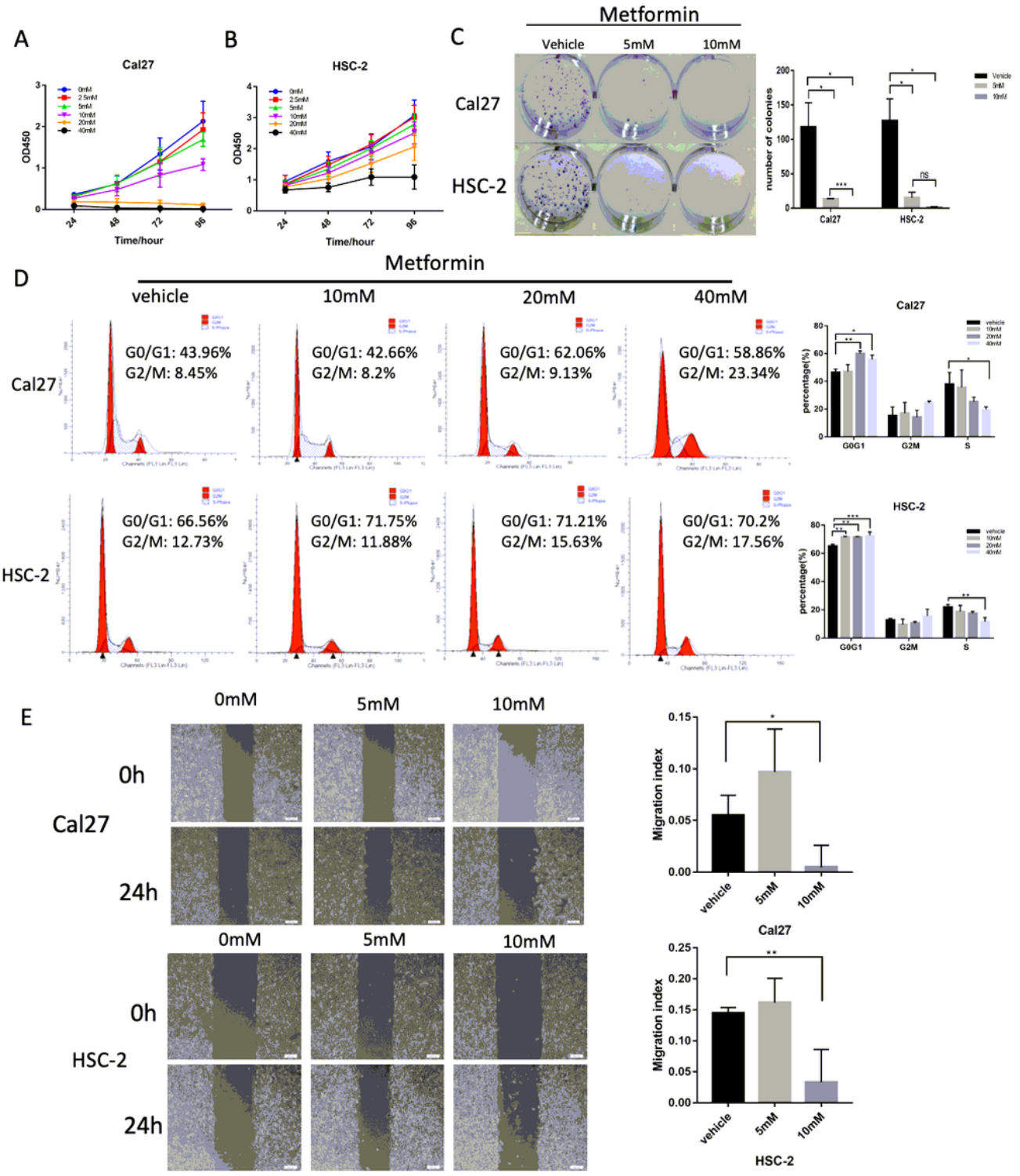

Figure 1

Metformin inhibited cell growth and induced cell cycle arrest of OSCC. (A, B) Cell viability of Cal27 (A) and HSC-2 (B) cells induced by metformin was evaluated by CCK8 assay. Cells were treated by concentration gradient of metformin (vehicle, 2.5, 5, 10, 20, and 40 mM) for 24, 48, 72, and 96 h. (C) Proliferative ability of cells treated by metformin (5 and 10 mM) was assessed by colony formation assay. Typical images (left) and quantification (right) in terms of colony numbers. (D) Cell cycle was detected by PI assay.

Typical images (left) and statistical analysis (right). (E) Cell migration ability was assessed by wound healing assay (left) and quantification (right) in terms of migration index. Data are presented as mean ± SD from three independent experiments at least. *: p<0.05; **: p<0.01; ***: p<0.001.

Figure 2

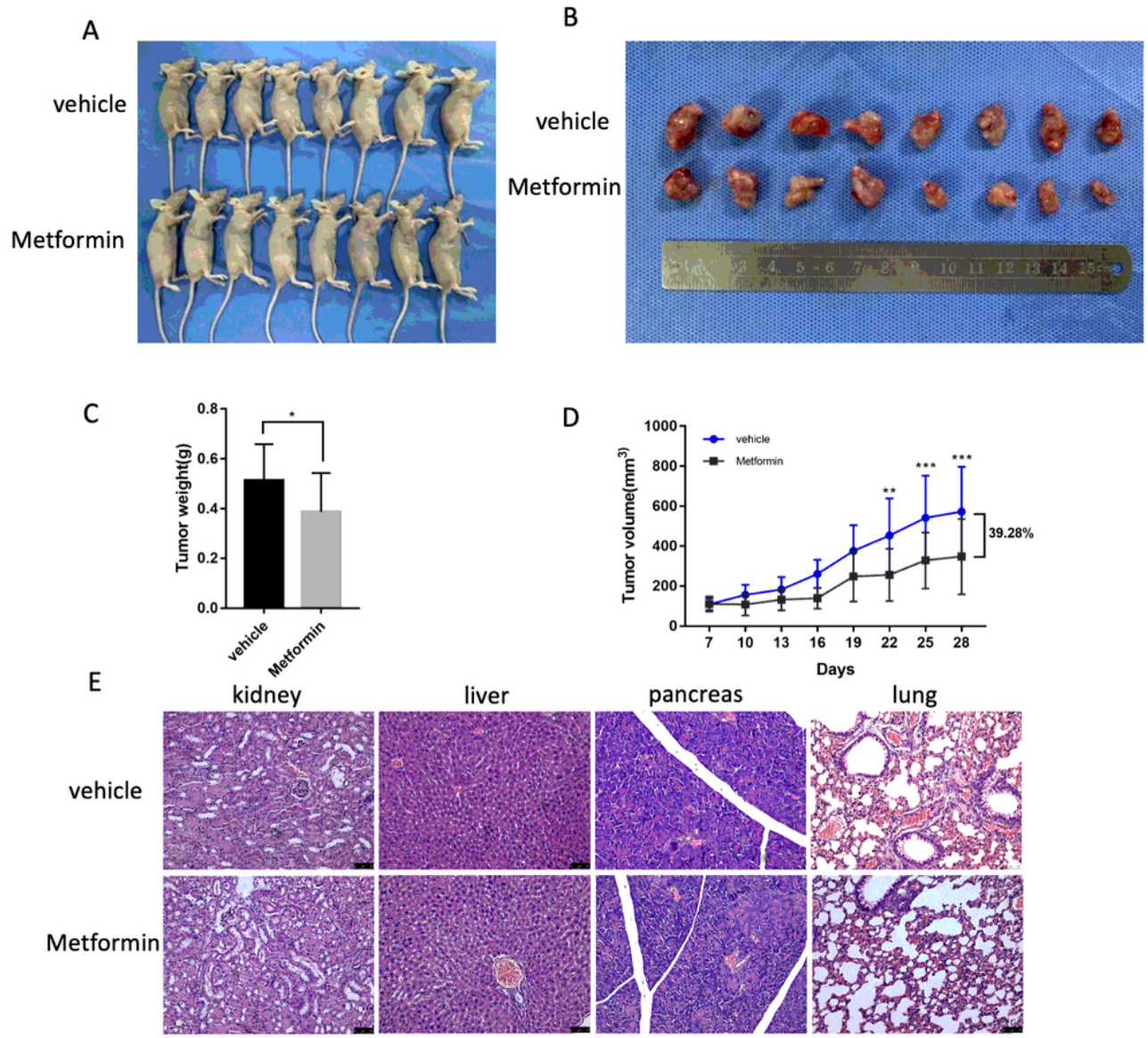

Figure 2

Metformin inhibited tumorigenesis of OSCC in vivo. (A) Nude mice at the end of the experiment. (B, C) Macroscopic appearance of the dissected tumors from mice at the end of the experiment (B) and tumor

weight histogram (C). (D) Graph indicated growth curves of the subcutaneously injected tumors treated by metformin and control group. (E) HE-stain was performed to indicate the histological characteristic of mice organs including kidney, liver, pancreas, and lung. The scale bars represent 100 μm. *: p<0.05; **: p<0.01; ***: p<0.001.

Figure 3

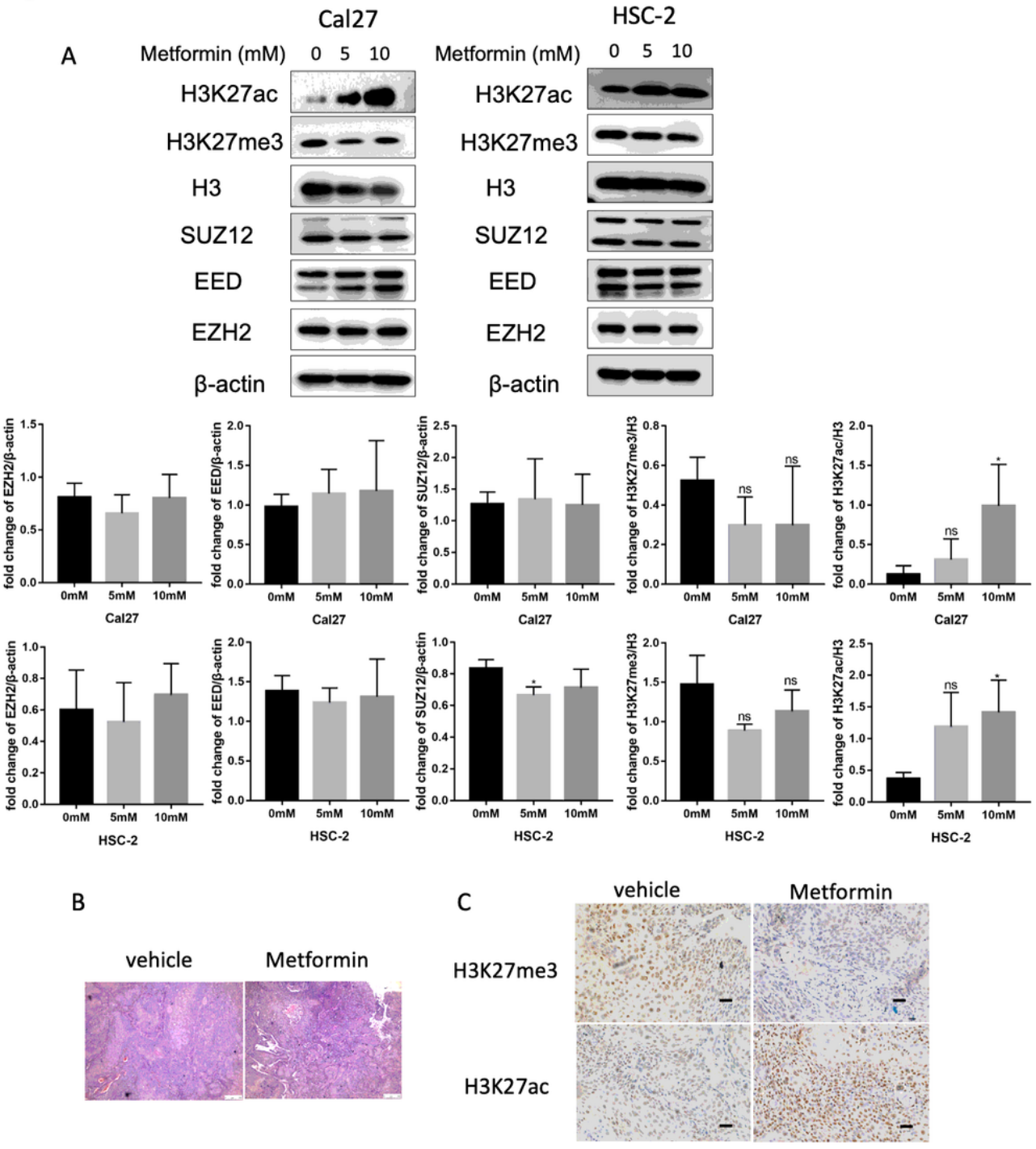

Figure 3

Metformin upregulated global level of H3K27ac and downregulated global level of H3K27me3. (A) OSCC cells treated with metformin (vehicle, 5 and 10 mM) for 6 days were subjected to western blotting analysis. H3 served as the reference protein for H3K27ac and H3K27me3, and β-actin was used as reference protein for EZH2, EED and SUZ12 (up). Histograms indicated the ratios of EZH2, EED, SUZ12 to β-actin, and ratios of H3K27ac and H3K27me3 to H3 (down). (B) Tumor tissues of nude mice were sectioned to conduct HE-stain (left) and immunohistochemistry stain (right) for antibody of H3K27ac and H3K27me3. The scale bars represent 100 μm. Representative figures were shown. Data are expressed as mean+SD from three independent experiments. ns: not significant; *: $p<0.05$.

Figure 4

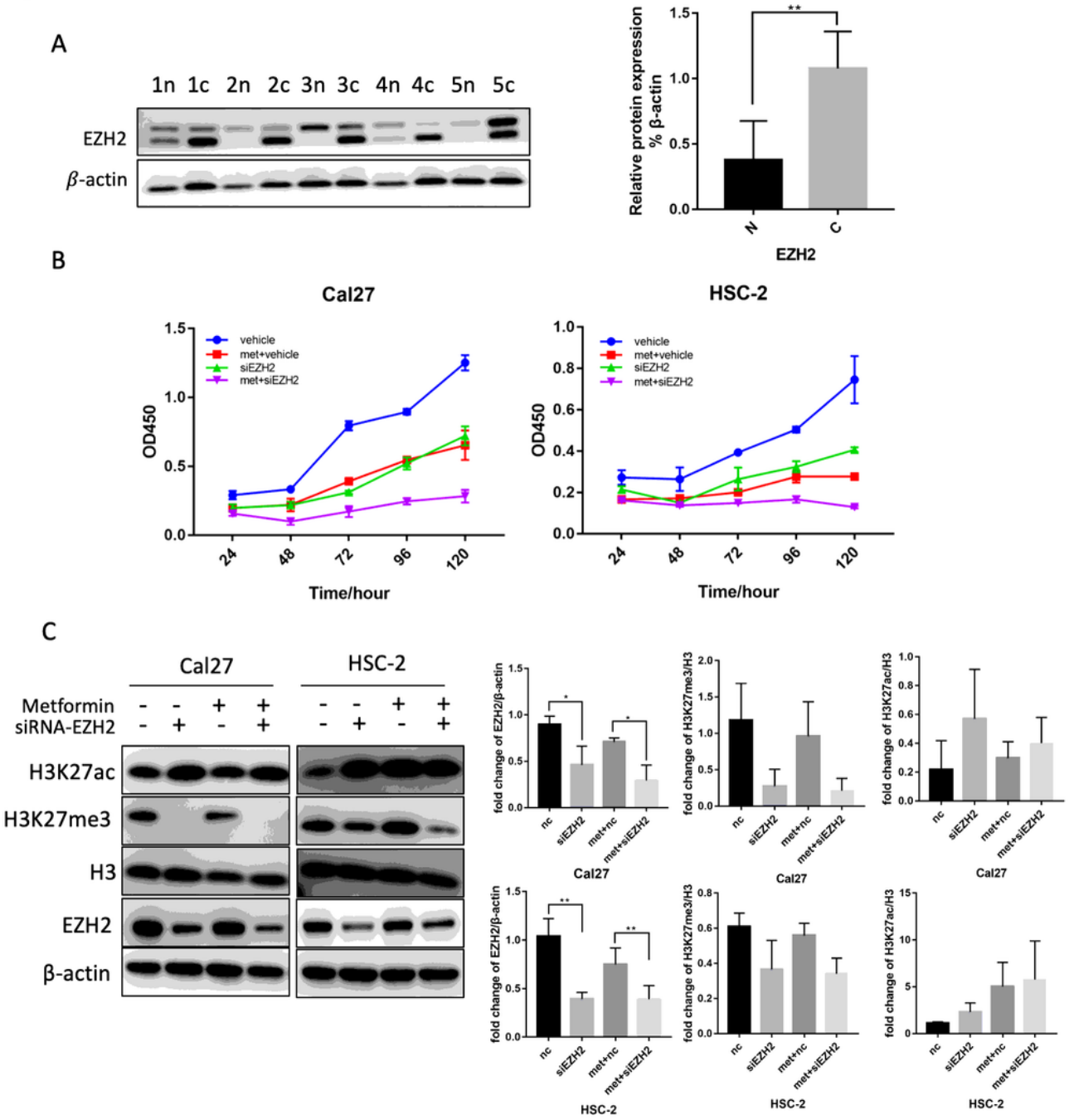

Figure 4

Metformin synergized with siRNA-EZH2 to enhance anti-cancer effect for OSCC. (A) EZH2 expression in OSCC tissues and adjacent normal tissues. Tissues were obtained from 5 patients with OSCC. The “C” indicated OSCC tissues, and “N” for adjacent normal tissues (left). Ratios of EZH2 to β-actin (right). (B) Cell viability of Cal27 (left) and HSC-2 (right) cells induced by metformin, siRNA-EZH2 alone or the combination was evaluated by CCK8 assay. Cells were treated by indicated interventions for 24, 48, 72,

96, and 120 h. (C) OSCC cells treated by metformin, siRNA-EZH2 alone or the combination were subjected to western blotting analysis (left). Ratios of EZH2 to β-actin, and H3K27ac and H3K27me3 to H3 (right). Representative figures were shown. Data are expressed as mean+SD from three independent experiments. **: p<0.05.

Figure 5

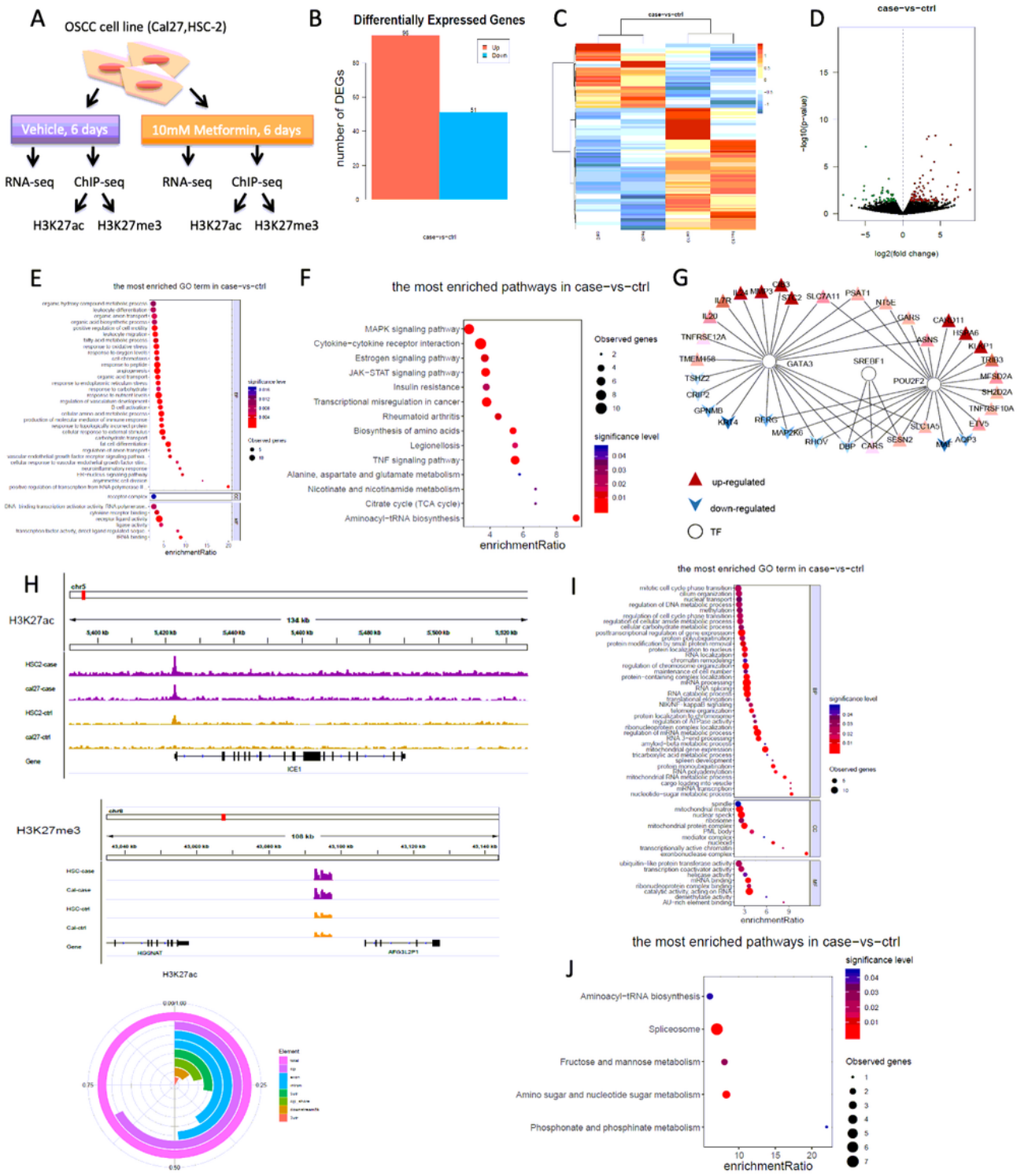

Figure 5

The genes associated with upregulation of H3K27ac induced by metformin was involved in the carcinogenesis and cancer metabolism regulation. (A) OSCC cell lines (Cal27 and HSC-2) were treated with vehicle control or 10 mM metformin for 6 days and then subjected to RNA-seq and ChIP-seq. (B) Overlap of the statistically (FDR $q < 0.05$) up- or down-regulated genes by metformin in the cell lines based on RNA-seq data. (C) Gene-wise hierarchical clustering heatmap indicated the enriched pathways in the oncogenic signatures from the metformin compared to vehicle control treated in Cal27 and HSC-2 cell lines. The color was based on FDR q value, and the darkest blue indicates $q \geq 0.1$ or N/A. (D) Volcano plot showed fold change versus adjusted p-values of metformin treatment and vehicle control. (E) The result of gene ontology (GO) analysis based on the RNA-seq data related to the differentially expressed genes. (F) The result of enrichment pathway analysis based on the RNA-seq data related to the differentially expressed genes. (G) The possible transcript factors analysis based on the RNA-seq data. (H) The 141 differential peaks associated with H3K27ac (up) and 1 differential peak associated with H3K27me3 (middle). The areas of differential peaks in the gene associated with H3K27ac (down). (I) The result of GO analysis based on the ChIP-seq data related to the differential peaks. (J) The result of KEGG analysis based on ChIP-seq data related to the differential peaks.