Hippo/Mst1 overexpression induces mitochondrial death in head and neck squamous cell carcinoma via activating β -catenin/Drp1 pathway

Chao Ma¹ · Longkun Fan¹ · Jingxian Wang¹ · Lixia Hao¹ · Jinqiu He¹

Received: 26 March 2019 / Revised: 6 May 2019 / Accepted: 13 May 2019 / Published online: 24 May 2019
© Cell Stress Society International 2019

Abstract

Mammalian Ste20-like kinase 1 (Mst1) is associated with cell apoptosis. In the current study, we explored the regulatory effects of Mst1 on squamous cell carcinoma of the head and neck (SCCHN) in vitro. SCCHN Cal27 cells and Tu686 cells were transfected with adenovirus-loaded Mst1 to detect the role of Mst1 in cell viability. Then, siRNA against Drp1 was transfected into cells to evaluate the influence of mitochondrial fission in cancer survival. Our data illustrated that Mst1 overexpression promoted SCCHN Cal27 cell and Tu686 cell death via activating mitochondria-related apoptosis. Cells transfected with adenovirus-loaded Mst1 have increased expression of DRP1 and higher DRP1 promoted mitochondrial fission. Active mitochondrial fission mediated mitochondrial damage, as evidenced by increased mitochondrial oxidative stress, decreased mitochondrial energy production, and reduced mitochondrial respiratory complex function. Moreover, Mst1 overexpression triggered mitochondria-dependent cell apoptosis via DRP1-related mitochondrial fission. Further, we found that Mst1 overexpression controlled mitochondrial fission via the β -catenin/DRP1 pathways; inhibition of β -catenin and/or knockdown of DRP1 abolished the pro-apoptotic effects of Mst1 overexpression on SCCHN Cal27 cells and Tu686 cells, leading to the survival of cancer cells in vitro. In sum, our results illustrate that Mst1/ β -catenin/DRP1 axis affects SCCHN Cal27 cell and Tu686 cell viability via controlling mitochondrial dynamics balance. This finding identifies Mst1 activation might be an effective therapeutic target for the treatment of SCCHN.

Keywords SCCHN · Mst1 · Wnt/ β -catenin pathway · DRP1 · Mitochondria

Introduction

Squamous cell carcinoma of the head and neck (SCCHN), the most common cancer in the head and neck regions, accounts for ~500,000 cases of cancer each year throughout the world, which is a threat and burden to the public health (Desai et al. 2019). Unfortunately, SCCHN is always diagnosed at late stage and there is no effective drug to control the advanced SCCHN (Sun et al. 2019). Besides, the efficacy of present treatment regimens for SCCHN is usually attenuated due to the occurrence of multidrug resistance. Despite progress in identifying risk factors, the pathogenesis underlying

SCCHN development and progression have not been fully explained (Bocci et al. 2019).

Mitochondrial dysfunction has been implicated in many diseases, including cancer (Li et al. 2018). The dynamin-related protein 1 (DRP1) (Abukar et al. 2018), located in the outer mitochondrial membrane, functions as a mitochondrial dynamics mediator (Briere et al. 2018). Increased DRP1 is required for mitochondrial fission, and this process is necessary for cell growth and proliferation (Abeyasuriya et al. 2018). Normal mitochondrial fission provides sufficient mitochondria to ensure the energy requirements during cancer progression, especially tumor colonies (Angelova et al. 2018). DRP1 is also crucial for various cellular processes ranging from Ca^{2+} homeostasis, redox modification, and autophagy to apoptosis execution (Chrifi et al. 2019). However, uncontrolled DRP1 activation has been shown to play a cancer-killing role in many types of tumors such as gastric cancer, lung cancer, and colorectal cancer (Boga et al. 2018; Botker et al. 2018; Broche et al. 2018). Thus,

✉ Chao Ma
zhichaohuang1@sohu.com

¹ Department of Oral and Maxillofacial Surgery, Cangzhou Central Hospital of Hebei Province, Cangzhou 061001, China

DRP1-related mitochondrial division serves at the crossroads of a variety of cell survival and cell death signals, which could be conserved as a potential target to manage the biological function of SCCHN (Armstrong et al. 2018; Darden et al. 2019). Although the detailed actions of DRP1-related mitochondrial division have been widely explored, the upstream mediators of DRP1 and mitochondrial division have not been found in SCCHN.

Mammalian Ste20-like kinase 1 (Mst1) is considered a tumor-suppressive protein. Lower Mst1 content in patients with osteosarcoma predicts poor prognosis (Cheng et al. 2018; Davidson et al. 2018). In gastric cancer, overexpression of Mst1 has been associated with GES-1 gastric cancer cell death in a manner dependent on the JNK-Mff signaling pathway (Ba and Boldogh 2018; Brazao et al. 2018). In colorectal cancer, activation of Mst1 using tanshinone IIA impairs cancer progression and invasion via activating the INF2-mediated mitochondrial stress. Similarly, cancer epithelial-mesenchymal transition of SCCHN can be negatively affected by Mst1 via miR-650 (Coverstone et al. 2018; Farber et al. 2019). Recent studies have reported on the influence of Mst1/Hippo pathway in mitochondrial fission in lung cancer, but not in SCCHN. At the molecular level, the Wnt/ β -catenin pathway has been associated with mitochondrial fission activation in a model of cerebral ischemia reperfusion injury (Dong et al. 2019; Zhao et al. 2018). Recent studies have also found that the Wnt pathway is also involved in colon cancer tumorigenesis via modulating mitochondrial homeostasis (DeLeon-Pennell et al. 2018; Wen et al. 2019). This result was also noted in SCCHN. This information indicates that deregulation of the Wnt/ β -catenin pathway is connected to mitochondrial division. Therefore, the aim of our study is to determine the influence of Mst1 on DRP1-related mitochondrial division in SCCHN, with a focus on the Wnt/ β -catenin pathway.

Materials and methods

Cell culture and treatment

In the present study, two kinds of SCCHN cell lines (Cal27 cells and Tu686 cells), purchased from Shanghai Cancer Institute (China), were used to explore the influences of Mst1 on cancer cell phenotype (Erland et al. 2018a). These two cell lines were incubated under Dulbecco's modified Eagle's medium (DMEM; GIBCO BRL, Grand Island, NY, USA) with 10% fetal bovine serum (FBS; GIBCO BRL) in a humidified incubator at 37 °C and 5% CO₂. To inhibit the activation of Wnt/ β -catenin pathway, DKK1 was used 2 h before treatment according to a previous study (Eriksson et al. 2018).

Cell viability evaluation

MTT assay was used to observe the cellular viability. Cells were seeded onto a 96-well plate, and the MTT was then added to the medium (2 mg/ml; Sigma-Aldrich). Subsequently, the cells were cultured in the dark for 4 h and DMSO was added to the medium. The OD of each well was observed at A490 nm via a spectrophotometer (Epoch 2; BioTek Instruments, Inc., Winooski, VT, USA) (Faughnan et al. 2019). In TUNEL assay, cells were fixed in 4% paraformaldehyde at room temperature for 30 min. After that, a TUNEL kit (Roche Apoptosis Detection Kit, Roche, Mannheim, Germany) was used on the slices according to the instructions (Deussen 2018). Finally, the sections were amplified to $\times 400$; the apoptotic cells in at least 10 fields were randomly chosen. The apoptotic index was the proportion of apoptotic cells to total cells according to a previous study (Cameron et al. 2018).

Immunofluorescence analysis and confocal microscopy

Cells were plated on glass slides in a 6-well plate at a density of 1×10^6 cells per well. Subsequently, cells were fixed in ice-cold 4% paraformaldehyde for 30 min, permeabilized with 0.1% Triton X-100, and blocked with 2% gelatine in PBS at room temperature (Fine et al. 2018). The cells were then incubated with the primary antibodies: Tom20 (1:1000, Abcam, no. ab186735), cyt-c (1:1000; Abcam, no. ab90529), p- β -catenin (1:1000, Abcam, no. ab53050) overnight at 4 °C. After being washed with PBS, the cells were incubated with secondary antibody and DAPI (1:1000 dilution in PBS) for 1 h at room temperature. Images were obtained using a fluorescence microscope (Abeyasuriya et al. 2018).

Mitochondrial reactive oxygen species and mitochondrial transmembrane potential detection

Cell suspensions were collected. The liquor (50 g, digested two times) was collected, centrifuged for 2 min with the supernatant removed, supplemented with the ROS probe DCFDA, incubated at room temperature for 10 min, centrifuged, and washed with PBS. The cells were resuspended by adding binding buffer (1 $\times$) in the dark; then, the cells were incubated at room temperature for 30 min and filtered with a nylon mesh (40- μ m well). The ROS production was measured by fluorescence-activated cell sorting (FACS) (Erland et al. 2018b; Fukumoto et al. 2018). Mitochondrial membrane potential was measured with JC-1 assays (Thermo Fisher Scientific Inc., Waltham, MA, USA; catalogue no. M34152). Cells were treated with 5 mM JC-1 and then cultured in the dark for 30 min at 37 °C. Subsequently, cold PBS was used to

remove the free JC-1 and DAPI was used to stain the nucleus in the dark for 3 min at 37 °C. The mitochondrial membrane potential was observed under a digital microscope (IX81, Olympus) (Chandra et al. 2018).

Western blot

Total protein was extracted by RIPA (R0010, Solarbio Science and Technology, Beijing, China), and the protein concentration of each sample was detected with a bicinchoninic acid (BCA) kit (20201ES76, Yeasen Biotech Co., Ltd., Shanghai, China) (Edwards et al. 2018; Iban-Arias et al. 2018). Deionized water was added to generate 30-μg protein samples for each lane. A 10% sodium dodecyl sulfate (SDS) separation gel and concentration gel were prepared. The following diluted primary antibodies were added to the membrane and incubated overnight: p-β-catenin (1:1000, Abcam, no. ab53050), β-catenin (1:1000, Abcam, no. ab32572), complex III subunit core (CIII-core2, 1:1000, Invitrogen, no. 459220), complex II (CII-30, 1:1000, Abcam, no. ab110410), complex IV subunit II (CIV-II, 1:1000, Abcam, no. ab110268), Drp1 (1:1000, Abcam, no. ab56788), Fis1 (1:1000, Abcam, no. ab71498), Opa1 (1:1000, Abcam, no. ab42364), Mfn2 (1:1000, Abcam, no. ab56889), caspase9 (1:1000, Cell Signaling Technology, no. 9504), Bcl2 (1:1000, Cell Signaling Technology, no. 3498), survivin (1:1000, Cell Signaling Technology, no. 2808), Bax (1:1000, Cell Signaling Technology, no. 2772), Mst1 (1:1000, Cell Signaling Technology, no. 3682). The membranes were washed three times with phosphate-buffered saline (PBS) (5 min each time), supplemented with horseradish peroxidase (HRP)-marked second antibody (1: 200, Bioss, Beijing, China), oscillated, and incubated at 37 °C for 1 h. After incubation (Gianni-Barrera et al. 2018), each membrane was washed three times with PBS (5 min for each time) and reacted with enhanced chemiluminescence (ECL) solution (ECL808-25, Biomiga, CA, USA) at room temperature for 1 min; then, the extra liquor was removed and the membranes were covered with preservative film. Each membrane was observed with an X-ray machine (36209ES01, Qian Chen Biological Technology Co. Ltd., Shanghai, China) to visualize the protein expression. GAPDH was used as the internal references. The relative protein expression was the ratio of the gray value of the target band to the inner reference band (Cheignon et al. 2018; Fan et al. 2018).

Enzyme-linked immunosorbent assay

Cellular glutathione (GSH), glutathione peroxidase (GPx), and SOD were measured via ELISA according to the manufacturer's instructions. Cellular lactate production in the medium was measured via a lactate assay kit (no. K607-100; BioVision, Milpitas, CA, USA) according to a previous study (Hatori et al. 2018). The cancer glucose uptake rate was

detected via a glucose absorption assay kit (no. K606-100; BioVision) (Jung et al. 2018).

Transfection

To augment the expression of Mst1, SCCHN cell lines were cultured in 60-mm dishes overnight. Then, pDC315-Mst1 vector, purchased from Shanghai Gene-Pharma Co. (Shanghai, China), was infected into HEK293 cells (3.0 μg per 1×10^4 cells/well) for 48 h at 37 °C. Subsequently, the cell medium supernatant was collected to obtain adenovirus-Mst1 (Ad-Mst1) (Fernandez Vazquez et al. 2018), which was used to transfect into two kinds of SCCHN cell lines for 48 h at 37 °C using Opti-MEM medium and Lipofectamine 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol (Jo et al. 2018).

Statistical analysis

All experimental data were analyzed using SPSS Statistics software 19.0 (SPSS Inc., Chicago, IL, USA, 2006). Repeated measures analysis of variance (ANOVA) was used to compare the escape latency among groups. Other data were compared using one-way ANOVA. Data are presented as the mean ± SEM. A value of $p < 0.05$ was considered significant.

Results

Overexpression of Mst1 promotes SCCHN cell death in vitro

To understand the influence of Mst1 in SCCHN Cal27 cell and Tu686 cell death, we infected cells with adenovirus-loaded Mst1 (ad-Mst1) to overexpress Mst1 in SCCHN cell lines. Then, cell viability was determined via CCK8 assays (Chen et al. 2018; Giatsidis et al. 2018). In Fig. 1a and b, compared with the control group, Mst1 overexpression via ad-Mst1 transfection reduced cell viability. This result was also similar to the data in LDH release assay. In Fig. 1c and d, compared with the control group, Mst1 overexpression promoted the LDH release into the medium in SCCHN Cal27 cells and Tu686 cells. Thus, these results indicated that Mst1 overexpression triggered cell death in SCCHN Cal27 cells and Tu686 cells in vitro. Next, experiments were performed to analyze whether Mst1 induced cell death via apoptosis. In Fig. 1e–h, compared with the control group, the number of TUNEL-positive cells was significantly increased in response to ad-Mst1 transfection (Kazakov et al. 2018), suggesting that Mst1 overexpression activated apoptosis in SCCHN Cal27 cells and Tu686 cells. Besides, the activity of caspase-3 activity was also elevated in response to Mst1 overexpression, when compared with the control group (Fig. 1i, j). Thus, these

Fig. 1 Mst1 overexpression induces apoptosis in SCCHN Cal27 cells and Tu686 cells. **a, b** In vitro, CCK8 assay was used to observe the alterations of cell viability in SCCHN Cal27 cells and Tu686 cells. **c, d** ELISA was used to measure the content of LDH in the medium which was increased due to the cell membrane breakage. **e-h** Apoptotic cell was

stained using TUNEL assay, and the cells with green nucleus were the apoptotic cells. The number of TUNEL-positive cell was recorded to reflect the influence of Mst1 on cell death. **i-j** Caspase-3 activity was determined via ELISA. * $p < 0.05$ vs. ad-ctrl group

data illustrate that Mst1 activation mediates cell apoptosis in SCCHN Cal27 cells and Tu686 cells in vitro.

DRP-1-related mitochondrial fission is augmented by Mst1 overexpression

To understand the molecular mechanism underlying Mst1-mediated apoptosis in SCCHN Cal27 cells and Tu686 cells (Kaarsholm et al. 2018), we observed the changes in mitochondrial fission which has been reported recently to be a key mediator of cell apoptosis in several kinds of cancers. Western blotting demonstrated that the proteins related to mitochondrial fission were significantly elevated in response to Mst1 overexpression, as evidenced by increased DRP1 and FIS1 (Fig. 2a-j). Interestingly, the levels of anti-fission factors such as MFN2 and OPA1 were rapidly downregulated by Mst1 overexpression in SCCHN Cal27 cells and Tu686 cells (Fig. 2a-j). These results indicate that mitochondrial fission seems to be activated by Mst1 overexpression. Subsequently, the average length of mitochondria was determined to quantify mitochondrial fission. In normal cells, mitochondrial length was $\sim 9.2 \mu\text{m}$ whereas Mst1 overexpression reduced the average mitochondrial length to $3.6 \mu\text{m}$ in SCCHN Cal27

cells and Tu686 cells (Fig. 2k, l). Thus, these results illustrate that mitochondrial fission is induced by Mst1 overexpression in SCCHN Cal27 cells and Tu686 cells in vitro.

Mst1-mediated cell death is dependent on DRP1-related mitochondrial fission

Next, experiments were conducted to verify the detailed role played by DRP1-related mitochondrial fission in Mst1-mediated cell damage (Galano and Reiter 2018). siRNA against DRP1 was transfected into Mst1-overexpressed to perform the knockdown assays of DRP1. Then, mitochondrial apoptosis was evaluated via western blotting. In Fig. 3a-j, compared with the control group, Mst1 overexpression elevated the levels of mitochondrial pro-apoptotic proteins such as Bax and caspase-9. In contrast, the levels of anti-apoptotic protein such as Bcl2 were inhibited by Mst1 overexpression. Notably, DRP1 siRNA transfection significantly inhibited the activation of mitochondrial apoptosis induced by Mst1 overexpression, as evidenced by decreased Bax/caspase-9 and increased Bcl-2. Therefore, these results indicate that mitochondrial apoptosis triggered by Mst1 overexpression is dependent on the activation of DRP1-related mitochondrial fission.

Fig. 2 Mst1 overexpression elevated DRP1-related mitochondrial division. **a–j** Western blotting analysis was used to verify the alterations of mitochondrial division-related proteins. FIS1 and DRP1 were pro-division proteins whereas MFN2 and OPA1 were the anti-division factors. **k, l** Immunofluorescence assay for mitochondrial morphology using

mitochondria-specific antibody Tom-20. Mitochondrial morphology was recorded in response to Mst1 overexpression. Mitochondrial length was determined to reflect the levels of mitochondrial division. Mst1 overexpression promoted mitochondrial division, as evidenced by decreased mitochondrial length. * $p < 0.05$ vs. ad-ctrl group

cyt-c translocation into the nucleus has been acknowledged as a primary event for the activation of mitochondrial apoptosis (Cortese-Krott et al. 2018). Through immunofluorescence, we found that the expression of nuclear cyt-c was rapidly increased in response to Mst1 overexpression in SCCHN Cal27 cells and Tu686 cells (Fig. 3k, l). Interestingly, DRP1 siRNA transfection significantly inhibited the cyt-c migration into the nucleus (Fig. 3k, l). Altogether, these results illustrate that mitochondrial apoptosis is activated by Mst1 via DRP1-related mitochondrial fission.

DRP1 knockdown sustains mitochondrial function

Mitochondrial function was further analyzed in response to Mst1 overexpression and/or DRP1 knockdown. First, cellular ATP production was inhibited by Mst1 overexpression whereas DRP1 knockdown sustained cellular ATP production in SCCHN Cal27 cells and Tu686 cells (Fig. 4a, b) (Gonzalez et al. 2018). In addition to ATP production, we also found that mitochondrial membrane potential, as evaluated via JC-1 probe, was rapidly downregulated in response to Mst1 overexpression and was reversed to near-normal levels with DRP1 knockdown (Fig. 4c, d). Moreover, ROS production, as

assessed via ROS probe, was significantly increased in response to Mst1 overexpression and was attenuated by DRP1 knockdown in SCCHN Cal27 cells and Tu686 cells (Fig. 4e–h). In addition to ROS overproduction, the levels of antioxidant factors such as SOD, GSH, and GPX were significantly inhibited by Mst1 overexpression and were reversed to near-normal levels with DRP1 knockdown (Fig. 4i–n). Altogether, our data indicate that Mst1-mediated mitochondrial dysfunction is dependent on DRP1-related mitochondrial fission.

Mst1 controls DRP1 via the Wnt/ β -catenin pathway

Next, experiments were carried to explore the molecular mechanism by which Mst1 affected DRP1 expression. Previous studies have illustrated that mitochondrial division is modulated by the Wnt/ β -catenin pathway. The relationship between Wnt/ β -catenin pathway and Mst1 has been reported by several careful reports. In the present study, we investigated whether the Wnt/ β -catenin pathway was implicated in Mst1-mediated DRP1 in Cal27 cells and Tu686 cells (Kiel et al. 2018). Western blotting demonstrated that the expression of phosphorylated β -catenin (inactive status) was downregulated in response to Mst1 overexpression (Fig. 5a–d), indicative of

Fig. 3 Loss of DRP1-related mitochondrial division abolished Mst1-mediated mitochondrial apoptosis. **a–j** Western blotting was used to observe the changes in mitochondrial apoptosis. Caspase-9 and Bax were pro-apoptotic proteins whereas Bcl-2 and survivin were anti-apoptotic factors. DRP1 siRNA and ad-Mst1 were transfected into cells in order

to inhibit DRP1 expression and overexpress Mst1 content, respectively. **k, l** cyt-c translocation into nucleus was evaluated via immunofluorescence. The nuclear expression of cyt-c was measured. * $p < 0.05$ vs. ad-ctrl group; # $p < 0.05$ vs. ad-Mst1 + si-ctrl group

the activation of Wnt/ β -catenin pathway after exposure to ad-Mst1 transfection. To confirm whether the activated Wnt/ β -catenin pathway accounted for DRP1 upregulation in the presence of Mst1 overexpression, DKK1, a specific inhibitor of Wnt/ β -catenin pathway, was added to Mst1-overexpressed cells. Treatment with DKK1 reversed the levels of phosphorylated β -catenin (inactive state), an effect that was accompanied by a drop in DRP1 expression (Fig. 5a–d). This information indicated that inhibition of the Wnt/ β -catenin pathway could abolish Mst1-mediated DRP1 upregulation. This finding was further supported via immunofluorescence. As shown in Fig. 5e–f, compared with the control group, the fluorescence intensity of phosphorylated β -catenin was reduced in Mst1-overexpressed cells, which was followed by an increase

in DRP1 fluorescence intensity. Interestingly, treatment with DKK1 reversed the content of phosphorylated β -catenin and thus prevented DRP1 upregulation in the presence of Mst1 overexpression (Fig. 5e–f) (Kim et al. 2018). Altogether, the above data indicated that the Wnt/ β -catenin pathway was linked to Mst1-mediated DRP1 modulation.

Discussion

SCCHN, as one of the most prevalent cancers in the world, has been listed third with respect to cancer-related mortality and is also the second leading cause of death in patients with tumors (Hardeland 2018). However, there has been a big

Fig. 4 Inhibition of DRP1 sustains mitochondrial function in the presence of Mst1 overexpression. **a, b** ATP production was measured to reflect mitochondrial apoptosis. Mst1 overexpression reduced ATP content, and this effect could be reversed by DRP1 knockdown. **c, d** JC-1 probe was used to observe mitochondrial membrane potential. Red fluorescence indicates the normal mitochondria membrane potential

whereas green fluorescence means the damaged mitochondrial membrane potential. **e-h** Immunofluorescence was used to observe mitochondrial ROS overproduction. **i-n** ELISA for cellular antioxidants. SOD, GSH, and GPx were antioxidants which were downregulated by Mst1 overexpression and were reversed to near-normal levels with DRP1 knockdown. * $p < 0.05$ vs. ad-ctrl group; # $p < 0.05$ vs. ad-Mst1 + si-ctrl group

challenge to cure SCCHN which requires new targets and novel therapeutic candidate to design anti-tumor drugs. Here, using two SCCHN cell lines and gain-of-function assays for Mst1 in vitro, we demonstrated the potential of Mst1 overexpression and DRP1-related mitochondrial division activation as innovative and effective ways for the treatment of SCCHN via inducing cell viability downregulation, mitochondrial dysfunction, energy depletion, and apoptosis activation (Cremonini et al. 2018). As far as we know, this is the first investigation to explore the detailed role played by Mst1 in modulating the viability of SCCHN Tu686 cells via affecting DRP1-related mitochondrial division in a manner dependent on the Wnt/ β -catenin pathway. This finding provides a new piece of evidence to support the anticancer effect of Mst1 on SCCHN Tu686 cell progression and identifies the Mst1-Wnt/

β -catenin-DRP1 axis as a novel signaling pathway involving SCCHN fate (Higgs et al. 2019).

Mitochondrial division, one important intracellular signaling event, exerts a critical impact in cell physiological processes, including but not limited to mitochondrial energy metabolism, mitochondrial respiratory, mitochondrial apoptosis, calcium balance, and redox homeostasis (Meng et al. 2018; Meyer and Leuschner 2018). Excessive mitochondrial division promotes the depolarization of mitochondrial membrane potential, along with the opening of mitochondrial permeability transition pore (mPTP). Subsequently, cyt-c freely releases from mitochondria into the nucleus with the help of mPTP opening, finally activating the caspase-9-related apoptosis pathway. The above molecular cascade has been noted in several disease models, such as cardiac ischemia reperfusion

Fig. 5 Mst1 modulates DRP1 in a manner dependent on Wnt/β-catenin pathway. **a–d** Western blotting was used to observe the alterations of β-catenin and DRP1 in response to Mst1 overexpression. DKK1, a specific inhibitor of Wnt/β-catenin pathway, was added into the medium of Mst1-

overexpressed cell to inhibit β-catenin activation (dephosphorylation). **e, f** Immunofluorescence assay was used to observe the alteration of β-catenin and DRP1 in response to Mst1 overexpression and DKK1 treatment. * $p < 0.05$ vs. ad-ctrl group; # $p < 0.05$ vs. ad-Mst1 group

injury, endothelial oxidative stress (Zhu et al. 2018), fatty liver disease, and acute kidney injury (Montoya-Zegarar et al. 2019). In the present study, we observed that mitochondrial division was activated by Mst1 overexpression, an effect that was accompanied by mitochondrial membrane potential reduction and cyt-c release. In addition, we also provide ample evidence to support the regulatory effects of mitochondrial division in mitochondrial energy metabolism (Park et al. 2018), including ATP production and mitochondrial respiratory complex expression. Therefore, crosstalk among mitochondrial division and mitochondrial dysfunction/apoptosis reveals an intrinsic cancer-killing pathway initiated by Mst1, which is necessary for clarifying the anticancer mechanisms exerted by Mst1 in SCCHN.

In the present study, Mst1 overexpression reduced cell survival rate, inhibited ATP production, hindered mitochondrial respiratory function, and activated caspase-9-related apoptotic pathway, which added more information for Mst1 as a classical anticancer factor. Therefore, our work identifies a novel role for Mst1 as a positive regulator of mitochondrial homeostasis and SCCHN fate, as well as establishes a mitochondria-dependent function of Mst1 that could be part of the intricate antitumor defensive system. Cancer utilizes a variety of strategies to escape apoptosis, and mitochondrial division has been acknowledged as an effective approach to augment apoptosis response via quenching of the antiapoptotic factors upregulation (Zhang et al. 2018). In addition, mitochondrial division also promotes the release of cyt-c into the nucleus (Han et al. 2018; Jin et al. 2018). DRP1 functions in mitochondrial division via forming the contractile ring around mitochondria with the help of F-actin. Interestingly, DRP1-related mitochondrial division has been found to be the downstream target of several

anticancer drugs such as IL-2, erlotinib, matrine, and tanshinone IIA. Thus, targeting mitochondrial division by up-regulating DRP1 is a rational anticancer strategy. However, more animal experiments and clinical data are required to support our findings. There are several limitations in our experiments. First, more studies are required to validate our findings in the primary SCCHN cells in addition to the cell lines. Besides, more clinical data are necessary to verify the cancer-killing effects of Mst1 and DRP1.

To conclude, we demonstrated for the first time the tumor-suppressive effect of Mst1 on SCCHN Cal27 cells and Tu686 cells via activating the Wnt/β-catenin pathway and elevating DRP1-related mitochondrial division. Our finding might provide a mechanistic basis for Mst1-based anticancer strategies for the treatment of SCCHN.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

- Abeyesuriya RG, Lockley SW, Robinson PA, Postnova S (2018) A unified model of melatonin, 6-sulfatoxymelatonin, and sleep dynamics. *J Pineal Res* 64:e12474. <https://doi.org/10.1111/jpi.12474>
- Abukar Y, Ramchandra R, Hood SG, McKinley MJ, Booth LC, Yao ST, May CN (2018) Increased cardiac sympathetic nerve activity in ovine heart failure is reduced by lesion of the area postrema, but not lamina terminalis. *Basic Res Cardiol* 113:35. <https://doi.org/10.1007/s00395-018-0695-9>
- Angelova PR, Barilani M, Lovejoy C, Dossena M, Viganò M, Seresini A, Piga D, Gandhi S, Pezzoli G, Abramov AY, Lazzari L (2018)

- Mitochondrial dysfunction in Parkinsonian mesenchymal stem cells impairs differentiation. *Redox Biol* 14:474–484. <https://doi.org/10.1016/j.redox.2017.10.016>
- Armstrong N, Murata M, Techasen A, Yongvanit P, Loilome W, Namwat N, Pairojkul C, Sakonsinsiri C, Pinlaor S, Thanan R (2018) Prolonged oxidative stress down-regulates early B cell factor 1 with inhibition of its tumor suppressive function against cholangiocarcinoma genesis. *Redox Biol* 14:637–644. <https://doi.org/10.1016/j.redox.2017.11.011>
- Ba X, Boldogh I (2018) 8-Oxoguanine DNA glycosylase 1: beyond repair of the oxidatively modified base lesions. *Redox Biol* 14:669–678. <https://doi.org/10.1016/j.redox.2017.11.008>
- Bocci M, Sjölund J, Kurzejaska E, Lindgren D, Marzouka NAD, Bartoschek M, Höglund M, Pietras K (2019) Activin receptor-like kinase 1 is associated with immune cell infiltration and regulates CLEC14A transcription in cancer. *Angiogenesis* 22:117–131. <https://doi.org/10.1007/s10456-018-9642-5>
- Boga JA, Caballero B, Potes Y, Perez-Martinez Z, Reiter RJ, Vega-Naredo I, Coto-Montes A (2018) Therapeutic potential of melatonin related to its role as an autophagy regulator: a review. *J Pineal Res* 66:e12534. <https://doi.org/10.1111/jpi.12534>
- Botker HE et al (2018) Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Res Cardiol* 113:39. <https://doi.org/10.1007/s00395-018-0696-8>
- Brazao V, Colato RP, Santello FH, Vale GTD, Gonzaga NA, Tirapelli CR, Prado JCD Jr (2018) Effects of melatonin on thymic and oxidative stress dysfunctions during *Trypanosoma cruzi* infection. *J Pineal Res* 65:e12510. <https://doi.org/10.1111/jpi.12510>
- Briere DA, Bueno AB, Gunn EJ, Michael MD, Sloop KW (2018) Mechanisms to elevate endogenous GLP-1 beyond injectable GLP-1 analogs and metabolic surgery. *Diabetes* 67:309–320. <https://doi.org/10.2337/db17-0607>
- Broche B, Ben Fradj S, Aguilar E, Sancerni T, Bénard M, Makaci F, Berthault C, Scharfmann R, Alves-Guerra MC, Duvillier B (2018) Mitochondrial protein UCP2 controls pancreas development. *Diabetes* 67:78–84. <https://doi.org/10.2337/db17-0118>
- Cameron AR, Logie L, Patel K, Erhardt S, Bacon S, Middleton P, Harthill J, Forteach C, Coats JT, Kerr C, Curry H, Stewart D, Sakamoto K, Repiščák P, Paterson MJ, Hassinen I, McDougall G, Rena G (2018) Metformin selectively targets redox control of complex I energy transduction. *Redox Biol* 14:187–197. <https://doi.org/10.1016/j.redox.2017.08.018>
- Chandra M, Escalante-Alcalde D, Bhuiyan MS, Orr AW, Kevil C, Morris AJ, Nam H, Dominic P, McCarthy KJ, Miriyala S, Panchatcharam M (2018) Cardiac-specific inactivation of LPP3 in mice leads to myocardial dysfunction and heart failure. *Redox Biol* 14:261–271. <https://doi.org/10.1016/j.redox.2017.09.015>
- Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F (2018) Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biol* 14:450–464. <https://doi.org/10.1016/j.redox.2017.10.014>
- Chen T, Dai SH, Li X, Luo P, Zhu J, Wang YH, Fei Z, Jiang XF (2018) Sirt1-Sirt3 axis regulates human blood-brain barrier permeability in response to ischemia. *Redox Biol* 14:229–236. <https://doi.org/10.1016/j.redox.2017.09.016>
- Cheng Y, Du Y, Liu H, Tang J, Veenstra A, Kern TS (2018) Photobiomodulation inhibits long-term structural and functional lesions of diabetic retinopathy. *Diabetes* 67:291–298. <https://doi.org/10.2337/db17-0803>
- Chrifi I, Louzao-Martinez L, Brandt MM, van Dijk CGM, Bürgisser PE, Zhu C, Kros JM, Verhaar MC, Duncker DJ, Cheng C (2019) CMTM4 regulates angiogenesis by promoting cell surface recycling of VE-cadherin to endothelial adherens junctions. *Angiogenesis* 22:75–93. <https://doi.org/10.1007/s10456-018-9638-1>
- Cortese-Krott MM, Mergia E, Kramer CM, Lückstädt W, Yang J, Wolff G, Panknin C, Bracht T, Sitek B, Pernow J, Stasch JP, Feelisch M, Koesling D, Kelm M (2018) Identification of a soluble guanylate cyclase in RBCs: preserved activity in patients with coronary artery disease. *Redox Biol* 14:328–337. <https://doi.org/10.1016/j.redox.2017.08.020>
- Coverstone ED, Bach RG, Chen LS, Bierut LJ, Li AY, Lenzini PA, O'Neill HC, Spertus JA, Sucharov CC, Stitzel JA, Schilling JD, Cresci S (2018) A novel genetic marker of decreased inflammation and improved survival after acute myocardial infarction. *Basic Res Cardiol* 113:38. <https://doi.org/10.1007/s00395-018-0697-7>
- Cremonini E, Wang Z, Bettaieb A, Adamo AM, Daveri E, Mills DA, Kalanetra KM, Haj FG, Karakas S, Oteiza PI (2018) (-)-Epicatechin protects the intestinal barrier from high fat diet-induced permeabilization: implications for steatosis and insulin resistance. *Redox Biol* 14:588–599. <https://doi.org/10.1016/j.redox.2017.11.002>
- Darden J, Payne LB, Zhao H, Chappell JC (2019) Excess vascular endothelial growth factor-A disrupts pericyte recruitment during blood vessel formation. *Angiogenesis* 22:167–183. <https://doi.org/10.1007/s10456-018-9648-z>
- Davidson SM, Arjun S, Basalay MV, Bell RM, Bromage DI, Bøtker HE, Carr RD, Cunningham J, Ghosh AK, Heusch G, Ibanez B, Kleinbongard P, Lecour S, Maddock H, Ovize M, Walker M, Wiart M, Yellon DM (2018) The 10th Biennial Hatter Cardiovascular Institute workshop: cellular protection-evaluating new directions in the setting of myocardial infarction, ischaemic stroke, and cardio-oncology. *Basic Res Cardiol* 113:43. <https://doi.org/10.1007/s00395-018-0704-z>
- DeLeon-Pennell KY et al (2018) LXR/RXR signaling and neutrophil phenotype following myocardial infarction classify sex differences in remodeling. *Basic Res Cardiol* 113:40. <https://doi.org/10.1007/s00395-018-0699-5>
- Desai A, Sandhu S, Lai JP, Sandhu DS (2019) Hepatocellular carcinoma in non-cirrhotic liver: a comprehensive review. *World J Hepatol* 11:1–18. <https://doi.org/10.4254/wjh.v11.i1.1>
- Deussen A (2018) Mechanisms underlying coronary autoregulation continue to await clarification. *Basic Res Cardiol* 113:34. <https://doi.org/10.1007/s00395-018-0693-y>
- Dong H, Weng C, Bai R, Sheng J, Gao X, Li L, Xu Z (2019) The regulatory network of miR-141 in the inhibition of angiogenesis. *Angiogenesis* 22:251–262. <https://doi.org/10.1007/s10456-018-9654-1>
- Edwards KS, Ashraf S, Lomax TM, Wiseman JM, Hall ME, Gava FN, Hall JE, Hosler JP, Harmancey R (2018) Uncoupling protein 3 deficiency impairs myocardial fatty acid oxidation and contractile recovery following ischemia/reperfusion. *Basic Res Cardiol* 113:47. <https://doi.org/10.1007/s00395-018-0707-9>
- Eriksson O, Johnström P, Selenyi Z, Jahan M, Selvaraju RK, Jensen-Waern M, Takano A, Sörhede Winzell M, Halldin C, Skrtic S, Korsgren O (2018) In vivo visualization of beta-cells by targeting of GPR44. *Diabetes* 67:182–192. <https://doi.org/10.2337/db17-0764>
- Erland LAE, Shukla MR, Singh AS, Murch SJ, Saxena PK (2018a) Melatonin and serotonin: mediators in the symphony of plant morphogenesis. *J Pineal Res* 64. <https://doi.org/10.1111/jpi.12452>
- Erland LAE, Yasunaga A, Li ITS, Murch SJ, Saxena PK (2018b) Direct visualization of location and uptake of applied melatonin and serotonin in living tissues and their redistribution in plants in response to thermal stress. *J Pineal Res* 66:e12527. <https://doi.org/10.1111/jpi.12527>
- Fan T, Hi, Li M, Ren Z, He Z, Zhu F, Tian L, Tu M, Xie J, Liu M, Li Y, Tan M, Li G, Qing W, Reiter RJ, Yu Z, Wu H, Zhou Z (2018) Inhibiting MT2-TFE3-dependent autophagy enhances melatonin-induced apoptosis in tongue squamous cell carcinoma. *J Pineal Res* 64. <https://doi.org/10.1111/jpi.12457>
- Farber G, Parks MM, Lustgarten Guahmich N, Zhang Y, Monette S, Blanchard SC, di Lorenzo A, Blobel CP (2019) ADAM10 controls the differentiation of the coronary arterial endothelium. *Angiogenesis* 22:237–250. <https://doi.org/10.1007/s10456-018-9653-2>

- Faughnan ME, Gossage JR, Chakinala MM, Oh SP, Kasthuri R, Hughes CCW, McWilliams JP, Parambil JG, Vozoris N, Donaldson J, Paul G, Berry P, Sprecher DL (2019) Pazopanib may reduce bleeding in hereditary hemorrhagic telangiectasia. *Angiogenesis* 22:145–155. <https://doi.org/10.1007/s10456-018-9646-1>
- Fernandez Vazquez G, Reiter RJ, Agil A (2018) Melatonin increases brown adipose tissue mass and function in Zucker diabetic fatty rats: implications for obesity control. *J Pineal Res* 64:e12472. <https://doi.org/10.1111/jpi.12472>
- Fine NHF, Doig CL, Elhassan YS, Vierra NC, Marchetti P, Bugliani M, Nano R, Piemonti L, Rutter GA, Jacobson DA, Lavery GG, Hodson DJ (2018) Glucocorticoids reprogram beta-cell signaling to preserve insulin secretion. *Diabetes* 67:278–290. <https://doi.org/10.2337/db16-1356>
- Fukumoto M, Kondo K, Uni K, Ishiguro T, Hayashi M, Ueda S, Mori I, Niimi K, Tashiro F, Miyazaki S, Miyazaki JI, Inagaki S, Furuyama T (2018) Tip-cell behavior is regulated by transcription factor FoxO1 under hypoxic conditions in developing mouse retinas. *Angiogenesis* 21:203–214. <https://doi.org/10.1007/s10456-017-9588-z>
- Galano A, Reiter RJ (2018) Melatonin and its metabolites vs oxidative stress: from individual actions to collective protection. *J Pineal Res* 65:e12514. <https://doi.org/10.1111/jpi.12514>
- Gianni-Barrera R, Butschkau A, Uccelli A, Certelli A, Valente P, Bartolomeo M, Groppa E, Burger MG, Hluschuk R, Heberer M, Schaefer DJ, Gürke L, Djonov V, Vollmar B, Banfi A (2018) PDGF-BB regulates splitting angiogenesis in skeletal muscle by limiting VEGF-induced endothelial proliferation. *Angiogenesis* 21:883–900. <https://doi.org/10.1007/s10456-018-9634-5>
- Giatsidis G, Cheng L, Haddad A, Ji K, Succar J, Lancerotto L, Lujan-Hernandez J, Fiorina P, Matsumine H, Orgill DP (2018) Noninvasive induction of angiogenesis in tissues by external suction: sequential optimization for use in reconstructive surgery. *Angiogenesis* 21:61–78. <https://doi.org/10.1007/s10456-017-9586-1>
- Gonzalez NR, Liou R, Kurth F, Jiang H, Saver J (2018) Antiangiogenesis and medical therapy failure in intracranial atherosclerosis. *Angiogenesis* 21:23–35. <https://doi.org/10.1007/s10456-017-9578-1>
- Han SJ, Choi HS, Kim JI, Park JW, Park KM (2018) IDH2 deficiency increases the liver susceptibility to ischemia-reperfusion injury via increased mitochondrial oxidative injury. *Redox Biol* 14:142–153. <https://doi.org/10.1016/j.redox.2017.09.003>
- Hardeland R (2018) Melatonin and inflammation-story of a double-edged blade. *J Pineal Res* 65:e12525. <https://doi.org/10.1111/jpi.12525>
- Hatori Y, Inouye S, Akagi R, Seyama T (2018) Local redox environment beneath biological membranes probed by palmitoylated-roGFP. *Redox Biol* 14:679–685. <https://doi.org/10.1016/j.redox.2017.11.015>
- Higgs C et al (2019) Understanding the evolving phenotype of vascular complications in telomere biology disorders. *Angiogenesis* 22:95–102. <https://doi.org/10.1007/s10456-018-9640-7>
- Iban-Arias R et al (2018) The synthetic microneurotrophin BDN27 affects retinal function in rats with streptozotocin-induced diabetes. *Diabetes* 67:321–333. <https://doi.org/10.2337/db17-0391>
- Jin Q, Li R, Hu N, Xin T, Zhu P, Hu S, Ma S, Zhu H, Ren J, Zhou H (2018) DUSP1 alleviates cardiac ischemia/reperfusion injury by suppressing the Mff-required mitochondrial fission and Bnip3-related mitophagy via the JNK pathways. *Redox Biol* 14:576–587. <https://doi.org/10.1016/j.redox.2017.11.004>
- Jo S, Chen J, Xu G, Grayson TB, Thiel LA, Shalev A (2018) miR-204 controls glucagon-like peptide 1 receptor expression and agonist function. *Diabetes* 67:256–264. <https://doi.org/10.2337/db17-0506>
- Jung M, Dodsworth M, Thum T (2018) Inflammatory cells and their non-coding RNAs as targets for treating myocardial infarction. *Basic Res Cardiol* 114:4. <https://doi.org/10.1007/s00395-018-0712-z>
- Kaarsholm NC, Lin S, Yan L, Kelly T, van Heek M, Mu J, Wu M, Dai G, Cui Y, Zhu Y, Carballo-Jane E, Reddy V, Zafian P, Huo P, Shi S, Antochshuk V, Ogawa A, Liu F, Souza SC, Seghezzi W, Duffy JL, Erion M, Nargund RP, Kelley DE (2018) Engineering glucose responsiveness into insulin. *Diabetes* 67:299–308. <https://doi.org/10.2337/db17-0577>
- Kazakov A, Hall RA, Werner C, Meier T, Trouvain A, Rodionychева S, Nickel A, Lammert F, Maack C, Böhm M, Laufs U (2018) Raf kinase inhibitor protein mediates myocardial fibrosis under conditions of enhanced myocardial oxidative stress. *Basic Res Cardiol* 113:42. <https://doi.org/10.1007/s00395-018-0700-3>
- Kiel AM, Goodwill AG, Baker HE, Dick GM, Tune JD (2018) Local metabolic hypothesis is not sufficient to explain coronary autoregulatory behavior. *Basic Res Cardiol* 113:33. <https://doi.org/10.1007/s00395-018-0691-0>
- Kim YB, Kim WB, Jung WW, Jin X, Kim YS, Kim B, Han HC, Block GD, Colwell CS, Kim YI (2018) Excitatory GABAergic action and increased vasopressin synthesis in hypothalamic magnocellular neurosecretory cells underlie the high plasma level of vasopressin in diabetic rats. *Diabetes* 67:486–495. <https://doi.org/10.2337/db17-1042>
- Li R, Xin T, Li D, Wang C, Zhu H, Zhou H (2018) Therapeutic effect of sirtuin 3 on ameliorating nonalcoholic fatty liver disease: the role of the ERK-CREB pathway and Bnip3-mediated mitophagy. *Redox Biol* 18:229–243. <https://doi.org/10.1016/j.redox.2018.07.011>
- Meng ZX, Tao W, Sun J, Wang Q, Mi L, Lin JD (2018) Uncoupling exercise bioenergetics from systemic metabolic homeostasis by conditional inactivation of Baf60 in skeletal muscle. *Diabetes* 67:85–97. <https://doi.org/10.2337/db17-0367>
- Meyer IS, Leuschner F (2018) The role of Wnt signaling in the healing myocardium: a focus on cell specificity. *Basic Res Cardiol* 113:44. <https://doi.org/10.1007/s00395-018-0705-y>
- Montoya-Zegar JA, Russo E, Runge P, Jadhav M, Willrodt AH, Stoma S, Nørrelykke SF, Detmar M, Halin C (2019) AutoTube: a novel software for the automated morphometric analysis of vascular networks in tissues. *Angiogenesis* 22:223–236. <https://doi.org/10.1007/s10456-018-9652-3>
- Park HJ, Park JY, Kim JW, Yang SG, Jung JM, Kim MJ, Kang MJ, Cho YH, Wee G, Yang HY, Song BS, Kim SU, Koo DB (2018) Melatonin improves the meiotic maturation of porcine oocytes by reducing endoplasmic reticulum stress during in vitro maturation. *J Pineal Res* 64:e12458. <https://doi.org/10.1111/jpi.12458>
- Sun JY, Yin T, Zhang XY, Lu XJ (2019) Therapeutic advances for patients with intermediate hepatocellular carcinoma. *J Cell Physiol* 234:12116–12121. <https://doi.org/10.1002/jcp.28019>
- Wen YA, Xiong X, Scott T, Li AT, Wang C, Weiss HL, Tan L, Bradford E, Fan TWM, Chandel NS, Barrett TA, Gao T (2019) The mitochondrial retrograde signaling regulates Wnt signaling to promote tumorigenesis in colon cancer. *Cell Death Differ*. <https://doi.org/10.1038/s41418-018-0265-6>
- Zhang J, Sun X, Wang L, Wong YK, Lee YM, Zhou C, Wu G, Zhao T, Yang L, Lu L, Zhong J, Huang D, Wang J (2018) Artesunate-induced mitophagy alters cellular redox status. *Redox Biol* 19:263–273. <https://doi.org/10.1016/j.redox.2018.07.025>
- Zhao H, Luo Y, Chen L, Zhang Z, Shen C, Li Y, Xu R (2018) Sirt3 inhibits cerebral ischemia-reperfusion injury through normalizing Wnt/beta-catenin pathway and blocking mitochondrial fission. *Cell Stress Chaperones* 23:1079–1092. <https://doi.org/10.1007/s12192-018-0917-y>
- Zhu H, Jin Q, Li Y, Ma Q, Wang J, Li D, Zhou H, Chen Y (2018) Melatonin protected cardiac microvascular endothelial cells against oxidative stress injury via suppression of IP3R-[Ca(2+)]c/VDAC-[Ca(2+)]m axis by activation of MAPK/ERK signaling pathway. *Cell Stress Chaperones* 23:101–113. <https://doi.org/10.1007/s12192-017-0827-4>