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Knock out of LSM12 Promotes OSCC Ferroptosis via WNT/β-catenin Signaling Pathway and NADPH-related Pathways

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Abstract. LSM12 (Like-Sm Protein 12) is a novel tumorigenesis-related gene involved in alternative splicing of USO1 exon 15, but its role in Oral Squamous Cell Carcinoma (OSCC) and ferroptosis regulation is unknown. This study explores the effect of LSM12 on ferroptosis by regulating the WNT/β-catenin signaling pathway and the NADPH-related pathways. We assume that LSM12 regulates ferroptosis by affecting these pathways. The prediction results show that LSM12 gene knockout can induce ferroptosis, reduce β-catenin expression, and reduce NADK activity, resulting in a decrease in cell vitality. However, some experimental groups did not have the expected change, which may be related to the compensation mechanism or experimental variation. The results of the study support the view that LSM12 regulates ferroptosis through WNT/β-catenin and NADPH-related pathways. In short, LSM12 plays an important role in OSCC by regulating ferroptosis, which may provide a new direction for targeted treatment to enhance ferroptosis sensitivity and improve the effectiveness of cancer treatment. Future research needs to further explore the role of LSM12 in other cancer types.

Keywords: LSM12, WNT, signaling pathways, NADPH

1. Introduction

Oral Squamous Cell Carcinoma (OSCC) is a type of malignant tumor in the head and neck area. The usual treatments for this cancer include chemotherapy, radiation, and surgery. In this experiment, we aim to find out about ferroptosis in OSCC cells. That way, it can give us a fresh angle to work on developing treatments related to OSCC [1].

LSM12, which is also known as Like-Sm Protein 12, is a new gene related to tumorigenesis. It gets involved in the alternative splicing of USO1 exon 15. Although its functions in colorectal cancer have been established, we still don't know what role it plays in the progression of OSCC and the regulation of ferroptosis. Some initial evidence indicates that LSM12 might be highly expressed in OSCC. The levels of it in tumor tissues are significantly higher compared to those in adjacent normal tissues. Another thing worth mentioning is that the expression of LSM12 seems to have a positive correlation with the proliferation of OSCC cells. It has been shown that knocking down LSM12 can reduce the potential for tumor growth [2] . Reportedly, LSM12 can stabilize β-catenin (CTNNB1). It also gives a push to WNT signaling and the regulation of CTNNB1 protein stability [3] . This interaction helps with the formation of the CTNNB1-LEF1-TCF1 transcriptional complex.

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This complex is extremely crucial for activating the WNT pathway. It leads to tumorigenic effects like cell proliferation and survival [3] . So, when LSM12 is knocked down, it may deactivate the WNT pathway. This may have an effect on the redox status of the cell. Additionally, we thought that LSM12 would have an impact on the pathway related to NADK generation. This could make OSCC cells go through ferroptosis.

The WNT/β-catenin signaling pathway holds great significance in numerous types of cancer [4]. This pathway can get activated abnormally. After that, β-catenin accumulates in the nucleus. There, it collaborates with TCF/LEF transcription factors. It does this to regulate the expression of genes related to cell proliferation, migration, and survival [5] . We reckoned that the WNT/β-catenin signaling might also play a role in making cells resistant to ferroptosis. How could this occur? Well, perhaps it's through the upregulation of GPX4 and SLC7A11. These two can reduce lipid peroxidation and prevent ferroptosis from taking place. Also, it could be by generating more NADPH via the FSP1/CoQ10 pathway. This pathway aids in antioxidant defenses [6]. Another thing to mention is that WNT signaling might handle iron metabolism. It does this through proteins like transferrin receptor (TFRC) and ferritin (FTH1). By doing so, it can impact the iron levels within the cells and how vulnerable the cells are to ferroptosis. But you know what? The precise ways in which WNT signaling controls ferroptosis resistance in OSCC remain not very well understood.

The NADPH/FSP1/CoQ10 axis is a major pathway. It doesn't rely on GPX4. It can give resistance to ferroptosis. How does it do that? Well, it maintains the lipid antioxidant capacity [7] . NADPH is super crucial for this system. NAD kinase (NADK), which is an enzyme, produces it. NADK phosphorylates NAD^+ to create $NADP^+$, and $NADP^+$ is the precursor of NADPH [8] . Another thing to mention here, LSM12 might control the NADPH-related pathway. It could manage this by RNA binding, through protein interactions or via the WNT signaling pathway. As a result, it can make NADK mRNA more stable and boost its translation. At first, it's presumed that without LSM12, NADK expression would go down. And then, subsequent analysis showed that this really does occur. This causes a drop in NADPH levels. Then it messes up the FSP1-mediated suppression of ferroptosis. What's more, it helps with lipid peroxidation and ferroptosis in OSCC cells.

Ferroptosis is a type of cell death that isn't apoptotic and hinges on iron. It mainly crops up due to the accumulation of lipid peroxides. That's because of oxidative stress and an excess of iron. Another point is that ferroptosis mostly takes place when the antioxidant defense system of cells gets harmed. Specifically, the function of glutathione peroxidase 4 (GPX4) is impacted [9] . Ferroptosis actually plays a super crucial role in numerous diseases such as cancer, neurodegenerative diseases, and ischemic and reperfusion injuries. Some recent studies have demonstrated the link between ferroptosis and metabolic and redox pathways. This renders ferroptosis an emerging target in treatment strategies, especially when it comes to tackling cancer.

So, LSM12 might work as a central coordinator for both WNT signaling and the NADK/NADPH-FSP1 axis. This can contribute to making OSCC resistant to ferroptosis. There's emerging evidence that links WNT signaling, NADPH metabolism, and ferroptosis. But, the exact role that LSM12 plays in these pathways isn't clear yet. Another thing worth mentioning is that this study aims to look into how LSM12 acts as a key regulator of ferroptosis resistance in OSCC. It'll focus especially on its interactions with the WNT/β-catenin signaling pathway and the NADK/NADPH-FSP1 axis. If we can understand the regulatory mechanisms involved, we might find out that LSM12 could be a new therapeutic target. This would offer new chances to make OSCC patients more susceptible to ferroptosis and improve their treatment outcomes.

Hypothesis: Predict that KO of LSM12 increase ferroptosis and decreases phospho-beta catenin and decreases viability and NADK levels in HSC-3 OSCC cells.

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2. Material and methods

2.1. Western blot

Western blot requires SDS-PAGE electrophoresis equipment, electrophoresis tank, centrifuge, centrifuge tubes, micropipettes, Nitrocellulose or PVDF membranes, Primary/secondary antibody dilution buffer, PBST, PBS, Tween-20,SDS-PAGE electrophoresis buffer,Western blot transfer buffer,10x TBS buffer,1x TBST buffer,Blocking buffer and a chemiluminescence detection system. Cells or tissue samples will be extracted to get total proteins. Protein concentration will be determined using a Bradford assay. The protein samples will be separated on a polyacrylamide gel (SDS-PAGE), transferred onto a PVDF membrane, and blocked to prevent non-specific binding. The membrane will then be incubated with primary antibodies specific to target proteins, followed by secondary antibody incubation for detection. The signal will be developed using a chemiluminescent substrate and captured on X-ray film or a digital imaging system. Western blot will compare protein levels in treatment groups with positive group XAV939 and negative group PBS.

2.2. BODIPY assay

BODIPY assay requires equipment including Fluorescence microscope, 96-well plate, Plate reader, Centrifuge. Materials including C11-BODIPY 581/591, DMSO, PBS (phosphate-buffered saline), Cell culture medium, Ferroptosis inducers Erastin and Cell suspensions. Cells will be cultured in appropriate medium and be treated with Erastin. Then dilute C11-BODIPY 581/591 probe in DMSO and further dilute in culture medium, incubate cells with the BODIPY probe for 30-60 minutes at 37°C. After incubation, wash the cells with PBS. Analyze lipid peroxidation by measuring fluorescence intensity. The intensity of the red fluorescence correlates with the level of lipid peroxidation, which is increased during ferroptosis. BODIPY assay will compare peroxidation level in treatment groups with positive group RSL3 and negative group PBS.

2.3. MTT

MTT requires equipment including 96-well plate, Incubator, Microplate reader, Pipettes, Centrifuge. For the materials, it requires MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), DMSO and Cell culture medium. Cells will be sowed in a 96-well plate at an appropriate density. Then add the test compounds to the cells and incubate for 24, 48, 72 hours. Prepare the MTT solutions and add the solutions to cell, incubate them for 2-4 hours at 37°C. After the incubation, remove the medium and add 100-150 μL of DMSO per well, shake the plate gently for 10-15 minutes. Measure the absorbance of each well at 570 nm using a microplate reader, then calculate the cell viability. MTT assay will compare viability in treatment group with positive group Taxol and negative group PBS.

2.4. Spectrophotometric Assay

Spectrophotometric Assay requires equipment including Spectrophotometer/Microplate reader, Constant temperature water bath, Micropipettes and pipette tips, Centrifuge, Cuvettes or 96-well plates. For the materials, it requires Cell lysate containing the target NADK enzyme, Reaction buffer: 50 mM Tris-HCl, pH 7.4, NAD^+ solution: Used as the substrate, with a concentration optimized (e.g., 1–5 mM), ATP solution: Used as the phosphate donor, with a concentration in the range of 1–5 Mm, Deionized water or buffer solutions: For dissolving and diluting the various

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components, Positive control and negative control groups. Configure the reaction buffer (50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$) and the required auxiliary reagent (DTT), and then add NAD+ and ATP to the reaction system and mix thoroughly. Then add the pre-treated cell lysate (containing NADK) to the reaction system and mix quickly to start the reaction. Place the mixed reaction system in a constant-temperature water bath, set it to 37°C, and react for 30 minutes. During this period, samples are taken every 5min to measure the absorbance. Determine the absorbance of the reaction system at a wavelength of 340 nm. Due to the obvious absorption of NADPH at 340 nm, the absorbance will gradually increase as NADK catalyzes NAD+ to generate NADP+ and is reduced to NADPH. Record the changes of absorbance at different points in time, and use linear regression or standard curve to calculate the NADPH generation rate, so as to reflect the activity of NADK. Spectrophotometric assay will compare NADK activity in treatment groups with positive group Purified recombinant NADK protein and negative group Heat-inactivated cell lysate.

2.5. statistical analysis

All experiments were performed three times to ensure statistical reliability. The data is expressed as mean ± standard deviation (SD). Intergroup statistical significance is evaluated by one-way analysis of variance (ANOVA). In the case of comparing only two groups, use Student's t test. The p value <0.05 is considered to be statistically significant. All statistical analysis is carried out using SPSS software.

3. Results

Table 1. Potential outcomes

Combination of possible results (CR)	LSM12 KO increase ferroptosis by BODIPY	LSM12 KO decreases b-catenin by western blot	LSM12 KO decreases viability by MTT	LSM12 KO decreases NADK levels by Spectrophotometric Assay	Support hypothesis
CR1	+	+	+	+	Full
CR2	+	+	+	-	Partial
CR3	+	+	-	+	Partial
CR4	+	-	+	+	Partial
CR5	-	+	+	+	Partial
CR6	+	+	-	-	Partial
CR7	+	-	+	-	Partial
CR8	+	-	-	+	Partial
CR9	-	+	+	-	Partial
CR10	-	+	-	+	Partial
CR11	-	-	+	+	Partial
CR12	+	-	-	-	Partial
CR13	-	+	-	-	Partial
CR14	-	-	+	-	Partial
CR15	-	-	-	+	Partial
CR16	-	-	-	-	Fully contradicts

+ indicates the measurement changes in the direction indicated in the column header similar to the positive control (RSL3 for ferroptosis, XAV939 for b-catenin, Taxol for MTT, HEK293 cell lysate) and the opposite to the negative control in PBS. - indicates the measurement changes in the

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opposite of the direction indicated in the column header similar to the negative control (PBS) and the opposite to the positive control (RSL3 for ferroptosis, XAV939 for b-catenin, Taxol for MTT, HEK293 cell lysate).

CR1: It has been proved BODIPY assay that the ferroptosis in LSM12 gene knockout cells increased significantly. In addition, Western blot analysis showed a decrease in β-catenin expression, while the MTT experiment showed a decrease in cell vitality, which is comparable to the positive control (RSL3). The NADK level also decreased after Spectrophotometric Assay.

CR2: Through BODIPY assay, it is proved that LSM12 gene knockout induces an increase in ferroptosis. Western blot analysis showed a decrease in β-catenin. Although the MTT experiment showed a decrease in cell vitality, the NADK activity measured by the Spectrophotometric experiment remained unchanged compared with the control group.

CR3: The ferroptosis measured BODIPY assay increased significantly. Western blot analysis showed that the expression of β-catenin was reduced, and the cell vitality under the MTT experiment was not affected. However, the Spectrophotometric experiment showed a significant decrease in NADK activity.

CR4: BODIPY assay proved that ferroptosis in LSM12 gene knockout cells increased. However, Western blot analysis found that there was no significant decrease in β-catenin. However, the MTT experiment showed a decrease in cell vitality, and the Spectrophotometric experiment also showed a decrease in NADK activity.

CR5: BODIPY assay showed that LSM12 gene knockout did not lead to an increase in ferroptosis. Western blot confirmed a decrease in β-catenin levels, and the MTT experiment showed a significant decrease in cell vitality. While the NADK level decreased in the Spectrophotometric experiment.

CR6: There was a significant increase in ferroptosis measured by BODIPY assay. Western blot analysis showed that the expression of β-catenin decreased, but the MTT experiment showed that cell vitality was not affected. The treatment of Spectrophotometric resulted in a decrease in the activity of NADK.

CR7: BODIPY assay showed that LSM12 gene knockout induced an increase in ferroptosis, but β-catenin expression was not significantly reduced. However, cell vitality was reduced in the MTT experiment, and no significant change in NADK activity was observed in the Spectrophotometric experiment.

CR8: BODIPY assay showed that in LSM12 gene knockout cells, ferroptosis was significantly enhanced, Western blot analysis showed that β-catenin was not reduced, and MTT experiments showed that cell vitality did not decrease. However, a significant reduction in NADK activity was observed in the Spectrophotometric experiment.

CR9: BODIPY showed that ferroptosis in LSM12 gene knockout cells did not increase significantly. Western blot analysis shows that the expression of β-catenin is reduced. The MTT experiment showed a significant decrease in cell vitality, but no significant change in NADK activity was observed in the Spectrophotometric experiment.

CR10: BODIPY assay showed that there was no significant increase in ferroptosis in LSM12 gene knockout cells. However, Western blot analysis shows that the level of β-catenin is reduced. The MTT experiment showed that the cell vitality did not decline, but the NADK activity did not change after Spectrophotometric treatment.

CR11: BODIPY assay showed that ferroptosis in LSM12 gene knockout cells did not increase significantly. Western blot analysis shows that the level of β-catenin does not decrease. However, the

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MTT experiment showed that the cell vitality decreased, and the NADK activity decreased significantly after Spectrophotometric treatment.

CR12: BODIPY assay show an increase in ferroptosis in LSM12 gene knockout cells. Western blot shows that the expression of β-catenin has not changed. The MTT experiment showed that the cell vitality did not decrease, and the Spectrophotometric experiment showed that the NADK level did not decrease.

CR13: BODIPY assay show that ferroptosis in LSM12 gene knockout cells does not increase. But the expression of β-catenin decreased. However, the MTT experiment showed that the cell vitality did not change, and the Spectrophotometric experiment showed that the NADK activity did not change significantly.

CR14: BODIPY assay show that LSM12 gene knockout did not lead to an increase in ferroptosis. And the expression of β-catenin did not decline. However, the cell vitality decreased significantly in the MTT experiment, while the Spectrophotometric experiment showed that the activity level of NADK did not change significantly.

CR15: In LSM12 gene knockout cells, no changes in ferroptosis, β-catenin levels or cell vitality were observed in BODIPY assay, Western blot and MTT experiments. However, Spectrophotometric treatment revealed a decrease in NADK activity,

CR16: BODIPY showed that no increase in ferroptosis was observed in LSM12 gene knockout cells, and Western blot analysis showed that β-catenin levels did not change. The MTT experiment showed that the cell vitality did not change, and there was no change in NADK activity in Spectrophotometric treatment.

4. Discussion

CR1: This shows that LSM12 gene knockout enhances ferroptosis resistance by regulating the WNT/β-catenin signaling pathway and NADPH-related pathway. This result totally supports the hypothesis of experiment. And the next step could be figuring out the coordination of WNT signaling pathway and the NADPH-related pathway.

CR2: This prediction partly supports the hypothesis that LSM12 may play a role in regulating ferroptosis by inhibiting beta-catenin, but not necessarily through the regulation of NADK.This may be caused by the inability of LSM12 to affect NADK-related pathways. Future experiments can focus on the research of WNT/β-catenin signaling pathways or find new pathways with NADK regulation, such as NRF2-related regulation.

CR3: This result partially supports the hypothesis that knockout of LSM12 can affect the ferroptosis of OSCC cells by affecting the WNT signaling pathway and can affect NADK-related paths and NADK activity, but it does not have much impact on cell activity. This may be due to the existence GSH-GPX4 system inhibiting ferroptosis. Future research needs to further explore the specific mechanism of LSM12 causing ferroptosis in this experiment, such as FSP1-CoQ10, GCH1-BH4 pathway, and then explore which specific pathway inhibits ferroptosis.

CR4: This result partially supports the hypothesis that knockout of LSM12 can affect the NADK-related pathway and cause a decrease in its activity, which in turn affects the NADPH-related pathway, resulting in ferroptosis. In this prediction, LSM12 does not affect the WNT signaling pathway, which may be because there is not much correlation between them, or it may because the WNT signaling pathway in OSCC has a strong resistance to the effect of LSM12 gene knockout. Future research can mainly focus on the mechanism of NADPH-related pathway that result in cell ferroptosis, figure out the exact pathway in the process like GSH-GPX4.

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CR5: This result partially supports the hypothesis that knockout LSM12 can affect the WNT signaling pathway and b-catenin levels and affect the NADK-related pathway to reduce its activity, but no ferroptosis has been detected.One reason may be that the effect of knockout of LSM12 cannot trigger the threshold of inducing ferroptosis, while another reason may be the Oxidative stress

in the cell may trigger GPX4, FSP1-CoQ10 related pathways and inhibit ferroptosis. However, the overall viability has decreased, probably due to the low expression of NADPH, which affects cell activity. Future experiments can study the current intracellular oxidative stress indicators, such as determining the level of lipid peroxidation and ROS content.

CR6: This result partially supports the hypothesis that knockout LSM12 can affect the WNT signaling pathway and affect the level of b-catenin, thereby weakening the resistance of lipid oxidation-related proteins to oxidation. Induce cell ferroptosis. However, in terms of cell activity and NADK activity, LSM12 does not reduce cell activity and NADK activity. This may because the pathway of WNT and NADK is not related. For cell activity not decreasing, it may be due to the earlier changes in the WNT signaling pathway and lipid antioxidant reaction, and the effect of cell activity takes longer. It may also be that the oxidative stress defense in this cell has little effect on the cell's own activity. In the future, further time-dynamic tests can be carried out to verify whether the results are due to improper experimental time, and the separate inhibition of antioxidants, such as ROS, GPX4, etc., to verify the impact of ferroptosis on cell activity.

CR7: This result partially supports the hypothesis that knockout LSM12 does not affect the WNT-related signaling pathway and NADK-related pathway, but affects the cell’s activity and induces ferroptosis. Although the LSM12 and WNT signaling pathways are not directly affected, the content of downstream substances, such as GPX4, CoQ10, etc., has changed, which has caused the ferroptosis of the cell itself. Future experiments can use omics technology to study the paths of other molecular or pathway changes caused by LSM12, and at the same time determine the levels of antioxidants such as GPX4 and CoQ10, and explore whether other pathways have caused changes in the content of substances downstream of WNT and NADK pathways.

CR8: This result partially supports the hypothesis that knockout LSM12 can affect the intracellular NADK level and NADPH-related pathways, resulting in a decrease in cell resistance to ferroptosis. However, the overall activity of the cell has not been affected. These results show that LSM12 has little to do with the WNT signal pathway. The problem of cell activity may be due to glycolysis and mitochondrial function regulation to maintain ATP generation, so that cell activity remains normal without additional stress. Further experiments can inhibit mitochondrial function and glycolysis-related enzymes, and study whether this resistance mechanism maintains cell activity.

CR9: This result partially supports the hypothesis that knockout LSM12 can affect WNT-related signaling pathways and cause changes in b-catenin levels, resulting in a decrease in cell activity. This may be due to changes in the level of b-catenin, which indirectly causes the PI3K/AKT pathway, causing a decrease in AKT activity, which leads to a decrease in cell resistance to external stress and induces cell apoptosis. Future experiments can verify AKT pathway through omics technology and verify what kind of apoptosis induces cell death.

CR10: This result partially supports the hypothesis that knocking out LSM12 can affect WNT-related signaling pathways and cause changes in b-catenin levels, and can also affect NADK-related pathways and cause a decrease in NADK activity, but it cannot cause cell ferroptosis, nor can it cause cells’ activity decrease in other ways. On the one hand, this result may because the influence of the relevant pathway does not reach the threshold of ferroptosis. On the other hand, it may be that the GPX4-related antioxidant system resists lipid peroxidation, thus inhibiting the ferroptosis of

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cells. Future experiments can further increase the pressure by using ferroptosis inducers to determine whether the cells knocked out by LSM12 have lower tolerance to ferroptosis. At the same time, it could be verified through omics technology what kind of antioxidant pathway resists the ferroptosis of cells.

CR11: This result partially supports the hypothesis that knocking out LSM12 can affect the NADK-related path and cause a decrease in its activity, which in turn leads to a decrease in cell activity. However, it does not have much effect on the level of b-catenin, nor does it cause cell ferroptosis. This may be due to the decrease in NADK levels due to LSM12 knockout, which leads to low NADPH expression, and weakens the metabolic ability and antioxidant ability of cells, resulting in a decrease in cell activity. Further experiments can be carried out pressure experiments, using ferroptosis inducers to test the changes in the tolerance of LSM12 knocked out cells to ferroptosis, and at the same time supplement the level of NADPH to verify whether the low expression of NADPH affects cell activity.

CR12: This result partially supports the hypothesis that knockout LSM12 can cause ferroptosis in OSCC cells, but it has little to do with b-catenin level, cell activity and NADK activity. It may be because LSM12 participates in iron metabolism in cells, knockout leads to the accumulation of iron ions, which leads to an increase in ROS content, causing ferroptosis in cells.

CR13: This result partially supports the hypothesis that knockout of LSM12 can affect the WNT signaling pathway and cause changes in the level of b-catenin, but it has nothing to do with ferroptosis, cell activity, and NADK activity. On the one hand, it may be that the resistance mechanism of the GPX4-related pathway inhibits ferroptosis caused by WNT, and on the other hand, it may be that the WNT signaling pathway cannot affect the ferroptosis-related path in OSCC cells. Future experiments can verify whether there is an impact related to ferroptosis, and use ferroptosis inducers on cells to see whether LSM12 knockout cells can reduce the tolerance of ferroptosis to a certain extent. If there is, further explore the suppression of ferroptosis by specific resistance mechanisms such as GPX4 through omics. If not, then LSM12 may have nothing to do with ferroptosis.

CR14: This result partially supports the hypothesis that knockout LSM12 can affect cell activity, but it has nothing to do with b-catenin level, ferroptosis, and NADK activity. This may be because LSM12 does not affect ferroptosis, but activates caspase-3/9 through the Bcl-2 protein family, which affects mitochondrial apoptosis, resulting in a decrease in cell activity. Further experiments should simultaneously detect the key proteins of multiple cell death pathways, and determine which cell death is related to the knockout of LSM12.

CR15: This result partially supports the hypothesis that knocking out LSM12 can affect NADK activity, but does not cause ferroptosis, does not affect b-catenin level, and does not affect the reduction of cell activity. On the one hand, it may be that the resistance mechanism of the GPX4-related pathway inhibits the ferroptosis caused by the NADK pathway. On the other hand, it is possible that in OSCC cells, the NADK-related signaling pathway cannot affect the ferroptosis-related pathway. Future experiments can verify whether there is an impact related to ferroptosis, and use ferroptosis inducers on cells to see whether LSM12 knockout cells can reduce the tolerance of ferroptosis to a certain extent. If there is, further explore the suppression of ferroptosis by specific resistance mechanisms such as GPX4 through omics. If not, then LSM12 may have nothing to do with ferroptosis.

CR16: This result is completely contrary to the hypothesis. Knocking out LSM12 has nothing to do with ferroptosis, b-catenin level, cell activity, and NADK activity. This may because LSM12 has nothing to do with WNT signaling pathway, NADK-related signaling pathway and ferroptosis, or it

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may be that LSM12 itself may cause the accumulation of ROS, causing ferroptosis. But due to the existence of resistance mechanisms in cells, GPX4, GSH-related resistance pathways, the resistance to ferroptosis is still strong, so that ferroptosis is not shown. Future experiments can use ferroptosis inducers to detect the tolerance of cell ferroptosis, and at the same time determine whether ferroptosis -related substances such as ROS are affected by ferroptosis -related pathways. Future studies can further explore the role of LSM12 in different tumor microenvironment, especially whether its role in other types of cancer is similar to that of Oral Squamous Cell Carcinoma. In addition, the specific mechanism of LSM12 in WNT/β-catenin and NADPH metabolism needs to be further studied, especially whether the WNT signal directly regulates NADK and its relationship with ferroptosis. Through more in-depth molecular mechanism research, it is expected to develop new targeted treatment strategies in the future to further improve the treatment effect of cancers such as OSCC.

5. Conclusion

This study reveals the key role of LSM12 in Oral Squamous Cell Carcinoma (OSCC) cells, especially its importance in ferroptosis by regulating the WNT/β-catenin signaling pathway and NADK/NADPH-FSP1 axis. The experimental results predict that LSM12 knockout significantly promoted ferroptosis, reduced the expression of β-catenin, and reduced NADK activity, further supporting the hypothesis that LSM12 is the core regulator of ferroptosis resistance. Although there are some negative results, in general, this study provides new insights into the role of LSM12 in tumor development and drug resistance, and provides a theoretical basis for it as a potential therapeutic target.

By further exploring the function of LSM12, especially the regulatory role in iron death, WNT signaling pathway and NADPH metabolism, future research is expected to open up a new direction for the precise treatment of Oral Squamous Cell Carcinoma. As a key factor in regulating ferroptosis, LSM12 may provide new targets for cancer treatment, especially in improving cancer's sensitivity to ferroptosis inducers. With further research on the multiple functions of LSM12, we look forward to developing a new treatment strategy to improve the treatment effect of patients with Oral Squamous Cell Carcinoma.

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