2DG Affects Liver Cancer Cell Ferroptosis By Affecting

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Abstract

Cancer cells are characterized by high proliferation but at the expense of enhanced metabolic rates. If the active oxygen content in the cell exceeds the average level, cancer cells activate antioxidant defenses against this increase. Ferroptosis occurs when glutathione levels are reduced due to a lack of cysteine in the cell. Ferroptosis is a new type of asymptomatic apoptosis. We chose to investigate the sensitivity of cancer cells to the effect of 2DG on ferroptosis. These include some effects of 2DG on intracellular NADPH solubility and NADPH's role in converting cystine to cysteine in cancer cells. The effect of cysteine on glutathione, the inhibitory effect of glutathione on ROS in cancer cells, and the accumulation of ROS can lead to ferroptosis in cancer cells.

Keywords: cancer, cell, Ferroptosis

1 Introduction

1.1 *Backgroud about 2D Ginhibiting NADPH production*

Cancer cells have abnormal proliferative abilities, can develop their own set of signaling pathways without being monitored by the immune system, and can even evade apoptosis. 2DG is a glucose analogue that interferes with cellular processes such as energy consumption and oxidative stress [1]. 2DG-6-P phosphorylated by 2DG is not metabolized further, but accumulates in cells, resulting in inhibition of NADPH production.As a supplier of glutathione and peroxidase for reduction reactions, NADPH can detoxify ROS and maintain the stability of intracellular oxidative potential and repair REDOX damage [2].The use of 2DG will lead to oxidative stress in mitochondria: 2DG-6-P can simply take an action in the first pathway in pentose cycle, NADP+generates one NADPH, and the product cannot continue to generate the second NADPH, which will lead to NADPH deficiency, thus destroying the ability of the cell to resist oxidative stress and leaving the cell in a state of oxidative stress.

1.2 *Backgroud of napdh and cysteine transport*

Cysteine plays an important role in maintaining redox homeostasis and can also be used as a rate-limiting precursor of glutathione. Glutathione is a tripeptide form of antioxidant.Normal intracellular cysteine production is usually via the sulfur transfer pathway or protein degradation. For cancer cells, cystine is absorbed extracellularly using the Xc-system composed of SLC7A11and SLC3A2. Cysteine is eventually reduced to cysteine by NADPH.Under the condition of oxidative stress, cystine reaction controlled by SLC7A11 can inhibit intracellular oxidation and ensure normal cell survival. Cysteine transporters direct the uptake of cysteine when cells undergo oxidative reactions, and cysteine exists as a covalent compound.Cysteine is divided into two cysteine molecules after cytoplasmic uptake [3].

1.3 *Backgourd of GSH and ROS*

Glutathione (GSH) is one of the important components of the intracellular antioxidant system [4]. In cancer cells, high levels of glutathione are required for scavenging excess reactive oxygen species (ROS) and detoxifying xenobiotics, as a regulator, the redox state of the cell can be altered by it.At the same time, GSH can help cells resist lipid peroxidation and damage caused by reactive oxygen. High levels of glutathione can be detected in tumor cells.

When the aerobic cells were damaged, the levels of active oxygen in the cells increased.Cell survival requires the participation of reactive oxygen, and the normal level of reactive oxygen is often needed to regulate the cellular pathways. The signal pathways regulated by reactive oxygen play an important role in cell survival.Therefore, abnormal signaling pathways can lead to eventual cell damage or death if excess active oxygen is present. By increasing the inducible adaptive response of ROS, such as maintaining up-regulation of antioxidant systems, cells can be restored to redox homeostasis, and irreversible cell damage can be prevented [5].

2 Hypothesis

We present a hypothesis that 2DG, acting on cancer cells, modifies and destroys the antioxidant capacity of cancer cells through NADPH, which affects the susceptibility of HCC to ferroptosis. Phosphorylated 2DG-6-P by 2DG is not further metabolized, but accumulates in cells, thereby inhibiting the production of NADPH, which is

an important energy source for the cystine-to-cysteine reaction, lacking cysteine It will lead to the inability to synthesize glutathione; once the content of glutathione is insufficient to inhibit the accumulation of ROS, it will cause cancer cells to be oxidatively destroyed and enter ferroptosis.

3 Method

3.1 *NADP+/NADPHlevels*

Human hepatocellular carcinoma cells were cultuvated by using 2DG (10mM) for 24 hours, and then washing with PBS twice for scraping and harvesting. After centrifugation for 5min, the cell precipitate was removed and transferred to the extraction buffer [6]. We treat the cells with a cup-shaped horn. The treated cell suspension was cultured by adding water at 0C and sonicated for 2min and centrifuged for 5min. The supernatant was taken and the NADPH content was measured by spectrophotometer and compared with the standard curve.

3.2 *Cysteine andCystine afectNADPH*

Grow up cell and measure NADPH and NADP+: Use glucose-free DMEM with 10% fetal bovine serum to grow up UMRC2, UMRC6 and RCC4 cells under 5% CO2. Cells would incubated overnight. They then would splitting and centrifuge in a buffer solution. The solution would be separated into two layers. The upper layer would be added to NADP-cycling buffer solution. Put it into 30Cdark environment, and then add glucose-6-phosphate. Use it to measure NADPH and NADPH+. Measure cell death: After cells have been treated correctly, they would be trypsinized. Then, use PBS to wash them, and stained with cold PBS.

3.3 *GSH measurement*

We treated hepatocytes with a 24-hour cultured 6-port plate. Hepatocytes were cultured in different concentrations of SO, MMSNs or MMSNs SO solution for 4 hours.Concentration gradients were 0,10, 25, 50ug/ml.After four concentrations of treatment, the pbs solution was used to wash the hepatocytes and repeated three times.Triton-x- 100 was chosen as the buffer for lysis hepatocytes.The hepatocyte was lysed with 40ml and put into a centrifuge to obtain the lysed buffer.After centrifugation, 100ul DTNB solution was added to the supernatant for 30 minutes.The glutathione content was obtained by determining the UV absorption of the mixed solution at 412 nm.The percentage of GSH in hepatocytes should be measured by referring to the data of GSH in untreated cells [7].

3.4 *Lipidperoxides Measurement (ROS)*

The contents of lipid peroxides in cells were determined by laser confocal scanning microscopy.Free SO and MMSN were added to the hepatocytes cultured overnight on a petri dish.After incubation for 6 h, C11BODIPY581/591 was stained and re-cultured for 30 min.Images in the cell fluorescence state can be obtained using CLSM devices.The lower the accumulation of lipid peroxide, the weaker the fluorescence effect.

3.5 *Ferroptosis characterization*

We used the MARTINI model and a membrane with a lipid component associated with ferroptosis. MARTINI requires the inclusion of new paramagnetic peroxide beads. Membranes with lipid composition include membranes with high levels of PUFA, phosphatidyl ethanolamine head groups, long-tailed fatty acids, and lipid peroxides. After measuring the expression of membrane properties in lipid composition, we found that changes in membrane composition could explain apoptosis induced by iron death. Changes in lipid membrane shape and curvature ratio promote the feasibility of oxidizing agents. As a result, the rate of membrane destruction is accelerated, and eventually cell death occurs.

4 Result

4.1 *NADP+/NADPH expectedlevels*

After 2DG treatment, the ability of HCC cells to metabolize peroxides is impaired because 2DG competes with glucose for metabolism in PPP. The results in the table 1 show that the level of NADPH decreases after the use of 2DG, and the NADPH used to induce glutathione in HCC cells is impaired and 2DG blocks NADPH production in cancer cellseading to oxidative stress state [8].

Table 1. NADP+/NADPHlevels (nmol/ mg) inliver cancer cels cultivatedunder the condition ofusing 2DG, glucosedeprivation.

Group	NADPH	NADPH+/NADPH
Control	17.9	6.8
2DG	2.3	29.5
Glucose deprivation	3.2	62. 1

4.2 *Expected Cysteine and Cystine*

The overexpression of SLC7A11 would not affect the level of cystine in cell under full of glucose environment. It means that the cystine would reduce to cysteine quickly after SCL7A11 coming into cell. There are decrease in the level of GSH and increase in GSSG, ROH under absence of glucose environment. The process which cystine across into the cells by SLC7A11 would consume NADPH. After SLC7A11 mediated cystine comes into cells, the cystine

would consume lots of NADPH and cause the large accumulation of cysteine and other disulfides [9].

4.3 *Ferroptosis characterization*

The area of each lipid increases and the membrane width decreases. Lipid diffusion increases while curvature remains unchanged. This implies further acceleration in a positive feedback loop driving iron-death active membranes [10].

5 Conclusion

2DG in the phosphorylated state hinders the first step in pentose oxidation, leading to a decrease in NADPH, which does not appear to be further metab olized in the pentose cycle, and also cannot be metabolized to form pyruvate. Thus, 2DG inhibits pyruvate and NADPH production, thus damaging the redox process, which is more frequent in cancer cells with radiation and oxidative str ess, which damage their antioxidant defenses.

In general, NADPH is selected to measure how sensitive cancer cells are to ferroptosis, and the lack of NADPH will promote the occurrence of ferroptosis in cells. The decrease in NADPH affects glutathione levels, ultimately reducing glutathione production. The decrease of glutathione peroxidase (GPX4) activity, the decrease of cellular antioxidant capacity, which result in lipid peroxidation and metabolic dysfunction, lipid ROS increases, thereby triggering ferroptosis.Taken together, 2dg can affect nadph, thereby altering cellular oxidative sensitivity and triggering ferroptosis. NADPH acts as a power source for the conversion of cystine to cysteine, and the reduction of NADPH content leads to ROS oxidation leading to cell ferroptosis.

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