Cancer Insight

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Research Progress on the Relationship Between Mitochondrial Deoxyguanosine Kinase and Apoptosis and Autophagy in Lung Adenocarcinoma Cells

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ABSTRACT

Lung cancer is the leading cause of cancer-related deaths. Non-small cell lung cancer (NSCLC) accounts for about 85% of all lung cancers, and lung adenocarcinoma is the most common NSCLC. Most patients with lung cancer eventually lead to local and metastatic recurrence, including many patients who have completely removed the primary tumor during surgery and have no noticeable metastasis. There are two different deoxynucleotide triphosphate (dNTP) libraries in eukaryotic cells. The de novo synthesis of dNTPs in the cytoplasm is coordinated with the cell cycle and reaches a peak in the S phase, thereby providing deoxynucleotides for the replication of genomic DNA. In contrast, the mitochondrial pool of dNTPs is maintained through the mitochondrial deoxynucleoside rescue pathway throughout the cell cycle and is essential for mtDNA replication. Mitochondria are vital cell powers in assimilation and catabolism. Oxidative phosphorylation (OXPHOS) of mitochondria is essential for the self-renewal of cancer stem-like cells in lung cancer, glioblastoma and leukemia. Thymidine kinase 2 (TK2) and deoxyguanosine kinase (DGUOK) are two mitochondrial deoxynucleoside kinases, which are responsible for the transport of pyrimidine and purine deoxynucleoside in mitochondria. Apoptosis and autophagy are important processes that regulate cell proliferation and death in normal cells and cancer cells. Inducing cancer cell apoptosis and autophagy is an

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ISSN 2972-3388
doi: 10.58567/ci01010004
Received 1 June, 2022; Accepted 16 June, 2022; Available online 20 June, 2022

effective means to treat malignant tumors. This review discusses the research progress of the relationship between mitochondrial deoxyguanosine kinase and lung adenocarcinoma cell apoptosis and autophagy.

Keywords: Lung adenocarcinoma; Mitochondrial deoxyguanosine kinase; Apoptosis; Autophagy

1 Introduction

Lung cancer is the most common malignant tumor in the world. Because of its complex etiological mechanism, the rapid development of the disease and the lack of effective drugs, it has become the main cause of cancer related mortality. Therefore, it is very important to find effective methods to delay the progress of lung cancer or treat the disease. Lung adenocarcinoma is the most common type of lung cancer. The proliferation and metastasis of lung adenocarcinoma is the key to disease progression. Overactivation of various cytokines, oxidative stress factors and signal pathways will promote the proliferation and metastasis of tumor cells.

Mitochondria are double membrane intracellular organelles containing soluble matrix and unique genome. They are the main source of high-energy phosphate molecule adenosine triphosphate (ATP), which is essential for all active intracellular processes [1]. Mitochondrial respiratory chain is the result of interaction between two physically and functionally separated genomes [nuclear DNA and mitochondrial DNA (mtDNA)]. Human mtDNA is 16.6 kb circular double stranded DNA containing only 37 genes. There are 24 mtDNA genes encoding RNA devices, including 2 ribosomal RNAs and 22 tRNAs, which participate in the in situ translation of 13 respiratory chain proteins encoded by other mtDNA genes [2]. The protein encoded by mtDNA is all subunits of respiratory complex I, III, IV and V, while subunits of complex II are completely nuclear coded [3]. Because the factors responsible for mtDNA maintenance and replication are coded by nuclear DNA genes, mutations in any of these factors may, in principle, affect the integrity of mtDNA, leading to qualitative or quantitative mtDNA molecular damage. The latter does not spread itself, that is, it does not spread through maternal inheritance, but is separated from Mendelian traits after nuclear gene mutation.

The pathogenesis of diseases related to mitochondrial structure and function changes has been more and more recognized, including the infection of the central and peripheral nervous system, skeletal muscle, bone marrow, endocrine and exocrine pancreas, kidney, myocardium, intestine and lung. Human mtDNA encodes 13 mitochondrial respiratory chain proteins essential to OXPHOS. The replication of mtDNA is crucial for cells to supplement damaged mitochondria and maintain mitochondrial function [4]. The dNTP library for mtDNA replication can be provided through de novo synthesis. TK2 and DGUOK are two mitochondrial deoxyribonucleoside kinases, which are responsible for the transport of pyrimidine and purine deoxyribonucleosides in mitochondria respectively [5]. TK2 and DGUOK are essential for the phosphorylation of antiviral and anti leukemic nucleoside analogues prodrugs. TK2 and DGUOK play an important role in the transformation of these prodrugs into active compounds. Affecting mitochondrial OXPHOS directly affects fatty acid oxidation, leading to cell death and fibrosis damage. This review discussed the research progress of the relationship between mitochondrial deoxyguanosine kinase and apoptosis, autophagy of lung adenocarcinoma cells.

2 Lung adenocarcinoma

Lung cancer is a major cause of cancer related mortality. According to histology, lung cancer can be

divided into two main subtypes: small cell lung cancer and NSCLC, accounting for 15% and 85% of all cases, respectively. NSCLC is further divided into three types: squamous cell carcinoma, adenocarcinoma and large cell carcinoma [6]. Squamous cell carcinoma accounts for 25 – 30% of all lung cancer cases. It arises from the early form of squamous cells in the airway epithelium in the central bronchus of the lung. The most common type of lung cancer is adenocarcinoma (lung adenocarcinoma), accounting for about 40% of all lung cancers. Lung adenocarcinoma develops from small airway epithelial type II alveolar cells, which secrete mucus and other substances [7]. Large cell (undifferentiated) cancer accounts for 5-10% of lung cancer. This type of cancer has no signs of squamous or glandular maturation. Therefore, the default diagnosis is usually made by excluding other possibilities.

2.1 Structure, function and genetics of mitochondria

ATP is produced by OXPHOS through the respiratory chain on the mitochondrial inner membrane. In this process, cofactors decrease (nicotinamide adenine dinucleotide [NADH] decreases, flavin adenine dinucleotide [FADH2] decreases and electron transfer flavoprotein decreases), the intermediate metabolism of protein and lipid (ETF) transfers electrons to complexes I and II and ubiquinone, and the electrons flow down the electrochemical gradient to complexes III, cytochrome C, and finally to complexes IV, Thus, the active proton (H^+) is generated and transferred from the mitochondrial matrix to the intermembrane space, so as to establish the electrochemical gradient [8, 9]. Under complex V, protons flow back to the mitochondrial matrix and the released energy is used to synthesize ATP.

The unique feature of mitochondria in mammalian cells is the existence of a unique genome, namely mtDNA, which has nothing to do with the nucleus. Both nuclear and mtDNA genes encode respiratory chain peptide components, and 13 essential polypeptides were synthesized from small 16.5 kb circular double stranded mtDNA [10]. On the contrary, nuclear genes encode more than 70 respiratory chain subunits, as well as a series of enzymes and cofactors needed to maintain mtDNA. These genes include DNA polymerase- γ (Polymerase gamma, POLG), TK2 and DGUOK [11,12]. MtDNA also encodes 24 transfer RNAs (t-RNAs) required for protein synthesis in mitochondria. An important feature of mitochondria is that almost all mtDNA comes from unfertilized oocytes [13-15]. Most researchers believe that, in fact, from the time of fertilization, the paternal mtDNA without sperm can survive into the fertilized egg, so the embryo only develops with the maternal mtDNA.

3 DGUOK mutation in lung adenocarcinoma

Adenocarcinoma of the lung usually contains heterogeneous mixtures of tissue growth patterns, which are classified as "mixed type". Although histologic features and marker expression are still the basis for clinical diagnosis, the latest advances in sequencing technology have enabled people to understand the heterogeneity of tumors and allow further subdivision of lung adenocarcinoma into molecular subsets based on the classification of so-called driver mutations. These mutations represent molecular changes essential for tumor initiation and growth. They can usually be detected in genes that control cell proliferation and

survival. Therefore, tumors may rely on the expression of these monomutated oncogenes to promote tumor growth and survival, which is also known as the concept of oncogene addiction. Because tumor cells depend on the abnormal activity or survival and proliferation pathways of specific mutant genes, their inactivation is usually sufficient to induce growth arrest or cell death. In the case of acute destruction of oncogene products, the apoptosis reaction observed in tumors is caused by the differential decay of several survival promoting and apoptosis promoting signals produced by oncoproteins. The imbalance between apoptosis promoting signals and survival promoting signals may trigger carcinogenic shock, which may eventually lead to tumor cell death.

Several mutations in DGUOK gene were found in patients with depleted mtDNA of lung adenocarcinoma, accounting for 10% to 15% of the cases. Mutations in the second gene encoding mitochondrial TK2 were found in about 20% of patients with severe myopathic diseases affected by mtDNA depletion. Thymidine kinase and deoxyguanosine kinase are part of the rescue pathway of pyrimidine and purine nucleosides, respectively. Together with previous observations on the pathogenicity of TP and ANT1 in MNGIE and multiple mtDNA deletion syndromes, this finding highlights the importance of mitochondrial dNTP libraries in the pathogenesis of Mendelian disease, which affects the integrity and copy number of mtDNA.

DGUOK mutation is detected in lung adenocarcinoma, which leads to increased activation of receptors and plays a carcinogenic role- α） The binding of can lead to conformational changes of epidermal growth factor receptor (EGFR) and homodimerization or heterodimerization with other members of the epidermal growth factor receptor family [16, 17]. Subsequently, with the help of adaptor proteins (such as SHC and GRB-2), cytoplasmic TK domains undergo autophosphorylation, which triggers downstream signal transduction pathways: RAS/RAF/mitogen activated protein kinase (MAPK) pathway; Phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway; Janus kinase (JAK)/signal transducer and transfer activator (STAT) pathway [18-20]. This stimulates mitosis, leads to cell proliferation and inhibits apoptosis. These pathways are critical for normal cell growth. EGFR can also be used as a stimulator for the growth of lung adenocarcinoma.

4 DGUOK induces apoptosis of lung adenocarcinoma cells

The up regulation of apoptosis activity is the main mechanism of many anti-tumor drugs. Caspase family plays an important role in the process of apoptosis. Apoptosis is initiated by activating death receptors on cell membranes or by the convergence of lethal signals on mitochondria. The activation of death receptors, including Fas/FasL and TRAIL-R, may stimulate Fas related death domain proteins, up regulate caspase-8 and caspase-10 levels, and finally trigger the apoptosis process [21, 22]. The aggregation of lethal signals on mitochondria may lead to the permeability of mitochondrial membrane and the release of cytochrome C. This cytochrome C activates apaf-1, which in turn splits caspase-9 proenzyme into an active dimer form, and finally triggers the apoptosis process [22]. DGUOK can induce cancer cell apoptosis through several different mechanisms.

4.1 DGUOK induces apoptosis of lung adenocarcinoma cells through PI3K/Akt pathway

PI3K/Akt signaling pathway plays an important role in the occurrence and development of lung cancer. The activation of PI3K/Akt signaling pathway enhances cell proliferation and inhibits cell apoptosis through different mechanisms. The upregulation of Akt promotes the phosphorylation of Bad protein and activates Bcl-2. In addition, Akt activation can activate downstream NF- κB signal pathway and regulate the expression of target genes that inhibit apoptosis [23-25]. In lung adenocarcinoma, Akt activation is still a poor prognostic factor. Researchers have studied the effect of DGUOK on PI3K/Akt signaling pathway in A549 cells. When A549 cells were treated with DGUOK for 24 hours, the results showed that DGUOK induced apoptosis of A549 cells, and the Ser473 and Thr308 phosphorylation sites were reduced. Phosphorylated GSK3 β (downstream substrate of Akt). In previous studies, GSK3 β There are two opposite views on the role of. Some studies show that GSK3 β The activation of GSK3 can inhibit the occurrence and development of tumors, while other studies have shown that GSK3 β It can prevent the development of tumor to further confirm GSK3 β Role of and GSK3 β Involved in DGUOK mediated apoptosis of A549 cells, siRNA inhibited GSK3 in A549 cells β Expression of; After transfection, GSK3 β At the same time, the effect of apoptosis was also significantly enhanced. DGUOK inhibits Akt and GSK3 β Apoptosis of A549 cells was induced.

4.2 DGUOK through NF- κ Apoptosis of lung adenocarcinoma cells induced by B signal pathway

More and more studies have shown that inflammation may be an important factor in the development of cancer. NF-κB signal pathway is related to inflammation and cancer. NF- κ B involves a group of transcription factors with specific DNA binding sequences. When the upstream signal is activated IκB kinase, IκB inactivated and finally with NF-κB Separation. Free NF- κ B is activated, transferred to the nucleus and regulates the expression of target genes related to cell proliferation or apoptosis, including IAP, Mcl-1, Bcl-2 and Bcl xl [26]. A549 cells were treated with DGUOK for 48 hours, and flow cytometry showed that the cells changed from living cells to early apoptosis. First, measure the activity of the promoter, and find that compared with the control group. The activity of B promoter decreased by 20%. Use TNF-α (NF-κB upstream signal), which indicates that RelA with GFP marker is transferred from cytoplasm to nucleus. However, after treatment with DGUOK, the mobilization of RelA was inhibited, and RelA remained in the cytoplasm. All these results indicate that DGUOK has lowered NF- κ B expresses and blocks nuclear transport, which may be a process of apoptosis mediated by DGUOK.

4.3 DGUOK induces apoptosis of lung adenocarcinoma cells through EGFR signal pathway

More than 10% to 40% of NSCLC patients have EGFR mutations. EGFR is associated with inhibition of cell proliferation, angiogenesis, invasion, metastasis and apoptosis. The binding of EGFR with ligand will activate EGFR and convert it into dimer. Dimerization causes autophosphorylation by stimulating downstream signal transduction. EGFR pathway involves three downstream pathways: MAPK pathway, PI3K/Akt pathway and JAK/STAT pathway [27, 28]. On the one hand, EGFR upregulates Akt and ERK1/2 levels, phosphorylates Bad protein, and then inhibits apoptosis. Gefitinib is an EGFR tyrosine kinase inhibitor, which can effectively alleviate this inhibition. On the other hand, Akt pathway can induce the phosphorylation of apoptosis promoting protein Bax and prevent it from transferring to the nucleus. After

treatment with DGUOK, apoptosis was induced in cells through cystatin dependent pathway, and the expression of EGFR, p-ERK, p-STAT3 and p-Akt was significantly down regulated with the apoptosis process. When incubated with DGUOK for more than 30 minutes, the dimerization of receptors continued to decrease. Moreover, DGUOK increased the proportion of endogenous EGFR on the cell surface in a time and dose dependent manner. The internalization of EGFR reduced the level of downstream signal pathways, and DGUOK treatment significantly reduced the expression of EGFR.

5 DGUOK and autophagy of lung adenocarcinoma cells

Autophagy is a process of intracellular degradation and recycling. Autophagy and apoptosis usually occur simultaneously in cells. Autophagy usually reduces the tendency of cell apoptosis, but it is not fatal. Cell survival under stress involves autophagy. The main mechanisms of this process mainly include mitochondria and selective reduction of the accumulation of pro apoptotic proteins, such as selective removal of active caspases. However, if the pressure exceeds the intensity threshold, apoptosis will inhibit autophagy through the cleavage of basic autophagic proteins mediated by cystatin. This indicates that the design of autophagy apoptosis crosstalk has broad pathophysiological significance. Appropriate regulation of cell response to stress and regulation of apoptosis and autophagy may be a promising method for the treatment of lung adenocarcinoma.

It is reported that DGUOK triggered the autophagy process of cells. After DGUOK treatment, the proportion of lysosomes and autophagosomes increased significantly [29]. Beclin-1 is considered as a positive regulator of autophagy, while Atg5 is a part of Atg12-Atg5-Atg16 complex, which is used to monitor the up regulation of autophagy. The conversion of LC3-T to LC3-U indicates autophagic activity, indicating that the levels of Beclin-1 and Atg5 proteins can be used to evaluate autophagic process. P62 is an autophagic receptor, which can connect ubiquitinated protein with LC3 and accumulate p62 when autophagy is inhibited. The results showed that, compared with the control group, the expression of LC3-U, Beclin-1 and Atg5 increased significantly after DGUOK treatment, while the expression of p62 decreased. These results suggest that DGUOK may affect the autophagic activity of A549 cells.

There may be a close relationship between autophagy and apoptosis [30]. DGUOK mediates the induction of apoptosis in lung adenocarcinoma cells. However, low concentration of DGUOK treatment increased the proportion of apoptotic cells to a minimum extent (from 1.3% to 8.2%), while the proliferation was significantly inhibited. To confirm whether autophagy can protect cells from apoptosis, as previously reported, it used autophagy inhibitors HCQ and DGUOK in A549 cells. Low concentration (50%) compared to HCQ alone (0.8%) or DGUOK alone (2.5% μ G/mL) After combined treatment of DGUOK and HCQ, the proportion of apoptotic cells increased significantly, indicating that DGUOK activated autophagy and apoptosis of A549 cells, and autophagy inhibitors effectively enhanced the role of DGUOK mediated apoptosis. The combination of DGUOK and autophagy inhibitor can significantly enhance the anti-tumor effect of DGUOK, which may be an effective treatment for lung cancer.

6 Summary and outlook

Lung adenocarcinoma accounts for about 40% of the total lung cancer and is the main cause of cancer related deaths worldwide. Obstruction, invasion and subsequent metastasis increase the incidence of recurrence and treatment failure, which is the main cause of lung adenocarcinoma related death. A better understanding of the molecular mechanism of lung adenocarcinoma cell progression is essential for developing effective therapies. DGUOK is involved in the development of lung adenocarcinoma and is positively related to the overall survival of lung adenocarcinoma patients. In terms of function, overexpression of DGUOK weakens the dryness, invasion and migration of lung adenocarcinoma cells in vitro and in vivo. Knocking down DGUOK or using DGUOK inhibitors promoted these malignant phenotypes. In mechanism, DGUOK passes PI3K/Akt channel, NF- κ B signal pathway and EGFR signal pathway promote apoptosis and autophagy of lung adenocarcinoma cells. DGUOK mediated apoptosis and autophagy may have some unknown mechanisms, which in turn provide new therapeutic targets and greatly improve the role of new drugs in the treatment of lung adenocarcinoma. With the development of research, based on the genetic or pharmacological targeting of DGUOK in lung adenocarcinoma cells, developing effective therapies has become a promising therapeutic strategy.

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