Research progress of the effects of mtDNA deletion on the biological behavior of tumor cells
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ABSTRACT
The writer summarizes the biological behavior of Mitochondrial DNA deleted cells (ρ⁰ cells) of multiple tumor cells to investigate the effects of mitochondrial DNA (mtDNA) deletion on tumor. Apoptosis, growth and invasion ability of tumor cells can be affected by mtDNA deletion. It can also induce the multidrugresistance phenotype of tumor cells. MtDNA deletion mainly affects the tumor in two aspects: energy metabolism and intracellular signal transduction. This study may lead to the development of anti-cancer drugs targeting mtDNA.

1. Introduction
Mitochondrial DNA-deficient cells (ρ⁰ cells) of various tumor cells were cultured and found to have different biological manifestations from parental tumor cells, suggesting that mitochondrial DNA (mtDNA) deletion can have an effect on the tumor. However, the specific effects of mtDNA deletion on tumor development and its mechanism also need to be further explored.

ρ⁰ cells are kind of cell line with its mitochondrial DNA deletion and no mitochondrial function. Considering the instability of the mtDNA compared to nuclear DNA[1], researchers successfully induce ρ⁰ cells in ethidium bromide induction and other methods. ρ⁰ cells rely on the energy generated by glycolysis to survive. They are pyrimidine and pyruvate-dependent, and could accept exogenous mtDNA playing a role[2]. The author summarized the biological performance of the ρ⁰ cells of a variety of tumor cells to explore the effect of the mtDNA deletion on the tumor.

2. Apoptosis
Most studies have shown that mtDNA deletion inhibits tumor cell apoptosis, but other studies have shown that mtDNA deletions make tumor cells more sensitive or have no significant effect on apoptosis. The specific reason is not clear. Some researchers attributed to the different cells[3].

2.1. mtDNA deletion inhibits the apoptosis of tumor cells
A large number of research results approve the above conclusions. Lingxian Long[4] and other researchers use chemotherapy drugs doxorubicin (DOX) on liver cancer SK-Hep1 cell line and its ρ⁰ cells to make induced apoptosis study and found that compared with the parent cells, ρ⁰ cell apoptosis rate decreased significantly. The expression of P-gp, Bax and Bcl-2 in ρ⁰ cells may be one of the mechanisms. ρ⁰ cells P-gp mitochondrial translocation can
exert efflux pump action to remove the drug from mitochondria and change chemotherapeutic drug subcellular distribution, reduce intracellular drug concentration, so that \( \rho^0 \) cells produce apoptosis resistance. Sun Hengwen\(^5\) established and identified the deletion of mtDNA in human HepG cell Hep G2 cell line. It was found that the \( a / b \) of \( \rho^0 \) Hep G2 cells was significantly lower than that of normal Hep G2 cells and the number of \( \rho^0 \) Hep G2 transmembrane cells was significantly increased. The anti-apoptotic ability and invasive ability of \( \rho^+ \)Hep G2 cells were improved. Li’s\(^6\) experiment confirmed that the tumor cells mtDNA radiation damage and tumor cell radiation sensitivity of the dose-effect relationship, which may be the mechanism of radiation-induced tumor cell apoptosis in Sun Hengwen experiments. Jacques C\(^7\) compared the cervical cancer He LaS3 cells and its \( \rho^0 \) cells on the apoptosis of the inducer induced by the actinic acid. They found \( \rho^0 \) cell apoptosis rate was significantly lower than its parental cells and the expression of intracellular death related Protein 3 of \( \rho^0 \) cells decreased significantly.

### 2.2 mtDNA deletion enhances the apoptosis of tumor cells

Liu Zongwen\(^8\) and others study the relationship between human esophageal squamous cell carcinoma EC9706 mitochondrial DNA and apoptosis. They found that in the presence of EB, mtDNA copy number decreased with cell division until the 12th day when mtDNA completely lost and became \( \rho^0 \)EC9706 Cells. They found that while the mtDNA copy number decreased gradually, the apoptosis rate gradually increased and the apoptosis rate of \( \rho^0 \) cells was significantly greater than its parental cells. Rommelaere\(^9\) induced osteosarcoma-derived \( \rho^0 \) cells with staphylococcal drugs and found that apoptosis-related DNA fragments have increased. Its protein kinase 3 activity increased in \( \rho^0 \) cells compared to mtDNA intact osteosarcoma cells. Meanwhile, cytochrome C release increased and protein Bcl-2 resistance to apoptosis expression decreased. Its catharsis B release also increased. All of these changes resulted in \( \rho^0 \) cells highly sensitive to apoptosis caused by staphylocytin and the apoptotic rate increased significantly. Other researchers\(^10,11\) observed the reactions of the same cell lines to staurosporine, ultraviolet radiation, cisplatin and similar results were obtained.

### 2.3 mtDNA deletion has no significant effect on the apoptosis of tumor cells

Bressan\(^12\) induced apoptosis of human ovarian cancer A2780 cell-derived \( \rho^0 \) cell line with anthracycline antitumor agent MEN10755. The results showed that anthracycline-induced apoptosis did not differ significantly in \( \rho^0 \)A2780 cell line and its parental cell A2780. It was found that although anthracycline antibiotics could make mtDNA cleavage, this damage had no effect on mitochondrial mRNA expression.

### 3. Growth and invasive ability

The current literature and experimental results do not get agreement. Some researchers reported that mtDNA deletion enhance the growth and invasion of tumor cells, while more research results show that tumor cells growth and invasive ability weakened.

#### 3.1. mtDNA deletion enhances the growth and invasion of tumor cells

He Yuqi\(^13\) investigated the effects of mtDNA deletion on the malignant phenotype of SK-Hep1 cells. MTT assay was used to determine the growth curve. Transwell assay was used to detect the invasive ability of cells. The growth rate and invasion ability of \( \rho^0 \)SK-Hep1 cells Significantly enhanced. Lingxian Long\(^14\) used the same method on human lung cancer 95C cells and human lung cancer 95D cells and their respective \( \rho^0 \) cell to research their growth and invasion ability. They found that human lung cancer \( \rho^0 \) cells have stronger colonies formation and invasion ability. More obvious growth and mitochondrial damage may be involved in the formation of malignant phenotype of lung cancer cells.

#### 3.2 mtDNA deletion reduces the growth and invasion of tumor cells

Jia Ma\(^15\)'s experiment showed that \( \rho^0 \)Namalwa cells were compared with the parents of Namalwa cells by MTT assay, cell cycle assay and Transwell assay. The results showed that growth ability and migration ability of \( \rho^0 \)Namalwa cells were weakened and that may be associated with intracellular ROS production and intracellular \( \text{Ca}^{2+} \) concentration decreased. Ray\(^16\) studied human T cell leukemia MOLT-4 cell \( \rho^0 \) cell line. They found that compared with the parent MOLT-4 cells, \( \rho^0 \)MOLT-4 cell proliferation is relatively slow and proliferation is weak. He also found more lactic acid was produced in the process of proliferation of \( \rho^0 \)MOLT-4 cells and the oxygen consumption and cytochrome c oxidase activity were significantly decreased. Electron microscopy showed a parallel ridge on the elongated mitochondria of the parent cells, while the mitochondria of \( \rho^0 \)MOLT-4 cells were found to be increased shape with a bubble and the ridge was twisted. Wu Xiaofang\(^17\) found that proliferation and invasive ability of the \( \rho^0 \) colorectal cancer cell was significantly weaker than its parental cells. They successfully make the \( \rho^0 \) cells of colorectal cancer cell lines SW480, HCT 116, HCT-8 with EB treatment. The results showed that the cell processes of \( \rho^0 \) cell lines were significantly slower than their respective parental cell lines. However, the percentage of the cell cycle was not significantly different from that of the parent cells. It was found that the reducing of the production of ROS in \( \rho^0 \) cells is one of the reasons. Wang Lei\(^18\) found that \( \rho^0 \) cell line of laryngeal JHUo11 cell is far weaker in its growth capacity and speed than its parent...
cells. He cultivate mtDNA deletion ρ₀ o11 laryngeal cancer cells through ethidium bromide sustained 90 d of the JHUo11 cells. He get the above conclusions by observing the proliferation and differentiation of cells and found ρ₀ o11 laryngeal cancer cells need to add exogenous pyruvate and uridine to maintain its survival.

4. Multidrug resistance

Tumor cells resistant to a chemotherapeutic drug and other drugs of different structures or action mechanism at the same time, is known as multidrug resistance (multidrugresistance, MDR). Tumor cell multidrug resistance is an important cause of tumor chemotherapy failure.

It was found that mtDNA deletion could induce the production of multidrug resistance phenotype in tumor cells. Chemotherapy drug doxorubicin(DOX), Adriamycin (ADR) and cisplatin (CDDP) were used by XianLong Ling[4] and RongJiu Tan[9] to investigate human hepatocellular carcinoma SK-Hep1 cells and ρ₀SK-Hep1 cells respectively. In contrast, it was found that IC₅₀ (50% cell growth inhibition drug concentration) and R₁ (resistance index) of ρ₀ cells were larger than their parents SK-Hep1 cells to these three chemotherapeutic drugs. ρ₀SK-Hep1 cells have significant resistance to Chemotherapy drugs, showing multidrug resistance. Sun Yulan[20] do research on human breast cancer MDA-MB-231 cells and its ρ₀ cells to examine their multidrug resistance biological performance. She used cisplatin/DDP), gemcitabine (GEM), paclitaxel (PTX), fluorouracil (5-FU) and pirarubicin (THP) on human breast cancer MDA-MB-231 cells and its ρ₀ cells. The relative survival rate of ρ₀ cells was higher than that of MDA-MB-231 cells under the same drug treatment conditions, that is, the relative resistance to chemotherapy drugs. But with the effect of low concentration of DDP and 5-FU, MDA-MB-231 cells were relatively resistant to drugs. YU suggest that ρ₀ cells from breast cancer can increase the drug resistance of ρ₀ cells to chemotherapeutic drugs by up-regulating the expression of multidrug resistance genes and translating P protein polysaccharide products[21]. Two investigators, Jacques C[22] and Singh KK[23], compared the reactivity of cephalosporin He LaS3 and its ρ₀ cells to staurosporine and doxorubicin. They found that ρ₀ cells were well tolerated to these two chemotherapeutic drugs. ρ₀ cells has multidrug resistance performance. They also found that ρ₀ cells do not produce drug resistance due to reducing intake of drugs, increasing emissions or changing the cell cycle.

5. The mechanism of mtDNA deletion affecting tumor

MtDNA deletion may alter the tumor progression by influencing the energy metabolism of tumor cells. Reznik[24] found that mtDNA copy number was strongly related to cell energy metabolism gene in multiple types of tumor tissue, such as fatty acid β-oxidation and branched chain amino acid metabolism. In the energy metabolism re-programming of tumor cells. The interaction between oxidative phosphorylation and glycolysis pathway determines the metabolic orientation of the cells. The changes in mtDNA copy numbers involved tend to alter the complete structure of mitochondria, thereby inhibiting its Oxidation and phosphorylation and other functions, which is one of the mechanisms of tumor cell energy metabolism reprogramming[25].

MtDNA deletion and abnormalities not only actively promote the energy metabolism of tumor cells reprogramming, but also promote cell malignant transformation and tumor progression by changing the intracellular signal transduction including ROS, Bcl-2/Bax and Ca²⁺ and so on. ROS increased in the mitochondrial respiratory chain damage caused by mtDNA abnormalities. ROS not only damage mitochondrial function by attacking nucleic acids, lipids, proteins, but also active antioxidant molecules such as glutathione and thioredoxin.However, this contribute to the occurrence and progression of the tumor[26]. ROS itself is also conducive to tumor cell proliferation, apoptosis resistance, chemotherapy resistance, invasion and metastasis. Bcl-2/Bax interacts with the mitochondrial outer membrane to regulate the release of apoptosis-related proteins and cytochrome c and the ratio of both plays an important role in the apoptosis or survival of tumor cells[27,28]. In addition, mitochondria were involved in the regulation of Ca²⁺ homeostasis. Accompanied by the mtDNA mutation, the intracellular Ca²⁺ concentration increased and the activation of protein kinase C (PKC), NF-kB and other signaling pathways led to metabolic reprogramming and transfer phenotype of tumor cells[29].

6. Prospect

ρ₀ cell line provide a good cell model for the study of the relationship between mtDNA deletion and the tumor. MtDNA deletion has a certain impact in a variety of biological performance of tumor cells. The study of this aspect will bring the new dawn to the tumor risk prediction, precancerous lesions screening and detection, clinical targeted therapy, prognosis and other aspects of tumour. This will also provide relevant experimental basis to mtDNA-based anti-tumor drug development, showing good application prospects.

References


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