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Investigating the effect of Alpha-ketoglutarate on the proliferation behavior of breast cancer cells in vitro

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Introduction

Breast cancer is the most common type of cancer among women worldwide and the second leading cause of death for women in the world. Genetic background, age, alcohol and tobacco consumption and overweight are the main causes of breast cancer. Breast cancer has different degrees according to the size and number of tumors, the treatment of this cancer in the early stages is about four times more successful than the more advanced and metastasized stages of this disease. Traditional treatment methods such as chemotherapy and radiotherapy, due to their high side-effects on the patient's body, require more research to find a more suitable method to treat or improve this disease.

Alpha-ketoglutarate (AKG) as an intermediate metabolite in the Krebs cycle has various effects in different cellular mechanisms such as fatty acid production, regulation of cell metabolism and amino acid production cycle. Also, it has been proven in various effects of AKG on increasing life span in *C. elegans* worms, increasing ovarian reserves, increasing protein and collagen synthesis and raising energy levels.

Examining the effect of this substance on different types of cancer showed that AKG has different effects on cancer cells' metabolism. For example, AKG regulates tumor size by participating in citrate production cycle which is the main fatty acid precursor. In addition, AKG is the main precursor of glutamine and glutamate, which are the main source of energy for cancer cells in anaerobic and stress conditions. Therefore, AKG can play a vital role in regulating metabolism and growth rate of cancer cells. In this study, the effect of AKG on proliferation of 4T1 breast

cancer cells was investigated in order to find a new method to treat or reduce the growth of these cells.

Material and Methods

4T1 cells were cultured with RPMI medium with 10 % FBS and kept in an incubator at 37°C and 5 % CO_2. 4T1 cells were treated with different concentrations of AKG (from 60 to 200 μM) based on recent researchs and the viability of cells was determined using MTT assay. Due to the lowest percentage of viability among all, 160 μM of AKG was selected as the appropriate concentration for other tests. The next experiments were performed in two groups; Test group (treated with 160 μM AKG) and control group by two biological replicates for each group. Aiming to find effects of AKG on the proliferation of 4T1 cells, the growth rate and doubling time of the 4T1 cells were investigated. Colony forming assay was used to evaluate the clonal expansion ability of 4T1 cells. Then, flow cytometry was used to investigate the effect of AKG on the cell cycle profile and the percentage of cells in each phase of the cell cycle. Finally, scratch assay was applied to analyze the migration potential of 4T1 cells in the both groups, which represents tumorigenesis ability of these cells.

Result and Discussion

According to the results of the MTT test, 160 μM with a survival rate of 66.14 % was determined as the appropriate concentration. Examining the cell growth curve in the test and control groups showed that treated 4T1 cells grew at a slower rate, comparing with control group. Furthermore, in examining the doubling time of the cell population, the cells of the test group needed more time to double their population, as determined 64 hours in test group and 52 hours in control group. The results of the colony forming assay showed that the number of colonies in the test group was 150 colonies, whereas 200 colonies formed in the control group. In addition, the size of colonies in test group was smaller compared to that of control group. Regarding cell cycle analysis by flow cytometry, higher percentage of cells were stopped in S and G2 phase in the treatment group, while the highest percentage of cells was in G1 phase in the control group. Finally, the results of the scratch assay revealed that the cell migration and thus the filling of the scratch area was slower in the test group compared to the control.

According to the results of the MTT test, it was observed that the concentration of 160 μM AKG decreased the viability of 4T1 cells by 34 %, which according to the results of Atlante research in 2018, this effect might be mediated by AKG dehydrogenase-dependent epigenetic regulation of TET and DNA demethylation. AKG exerted an inhibitory effect on the growth of 4T1 cells. According to a study by Katarzyna in 2019, this effect of AKG is due to the effect is mediated by regulation of DNA replication and histone methylation. Atlante and colleagues also confirmed that AKG decreased the clonal expansion ability in colon cancer cells by increasing intracellular nitric

oxide (NO) and regulating TET enzyme, which is consistent with the results of this study. The results obtained by Abla and colleagues in 2020 showed that AKG regulates the transition of cells from G1 to S cycle and prevents the growth of cancer cells and entering to the division phase. They revealed that AKG exerts its effect on cell cycle profile by regulating the expression of cell cycle regulatory proteins such as cyclins and p21. Finally, according to Atlanta's study it was found that AKG reduced the ability of osteosarcoma cancer cells to metastasize by regulating matrix metalloproteinase and increasing intracellular nitric oxide, which were also consistent with the results of the scratch repair test.

Conclusions

The results showed that AKG reduced the growth and metastasis in 4T1 cancer cells by different mechanisms including regulation of DNA replication, methylation of histones and epigenetic regulation. Taken together, AKG might be used as a natural metabolite to treat breast cancer, as well as to reduce the chance of breast cancer development.

Keywords: Apoptosis, Alpha-ketoglutarate, Scratch assay, Cell cycle, Viability, 4T1 cell line, Colony formation.