ISSN: 2329-6119 (Print)
ISSN: 2329-6100 (Online)

International Journal of Life Science Study (IJLSS)

DOI: http://doi.org/10.7508/ijlss.01.2024.08.11

ARTICLE

EXPLORE THE ROLE OF DAPAGLIFLOZIN IN REGULATING THE KLF4-NDRG2 SIGNALING PATHWAY DURING THE DEVELOPMENT AND PROGRESSION OF COLON CANCER

Jinbo Wu[1], Zhiqiang Su[1], Liyi Qin[1], Cunxiu Ma[1], Lu Wang[1], Xiaojuan Li[1,2*]

[1] Northwest Minzu University, Lanzhou 730000, China
[2]The second people's Hospital of Gansu province, Lanzhou 730000, China
[*]Correspondence Author E-mail: 875501566@qq.com

ARTICLE DETAILS

Article History:

Received 2 January 2024
Accepted 21 March 2024
Available online 25 April 2024

ABSTRACT

Objective: The aim of this study was to investigate the regulatory effect of dagliflozin on the KLF4-NDRG2 signaling pathway in colon cancer cells, and to clarify the effect of dagliflozin on the process of colon cancer cell development. Methods: 10 colon cancer RKO cell strains were divided into control group and experimental group, the starting time of the study was July 2023, and the ending time was October 2023, the number of cells in each group was 5. The cells in the control group were applied to spread RKO cells into 6-well plates, and the cell strains in the experimental group were applied to pre-stimulation with 2 **μm** dagliflozin, and the improvement of the lineation of the two cell strains was observed by taking the same field of view, while the migration distance of the cells in the control group was calculated and compared with that of RKO cells. At the same time, the cell migration distance was calculated to compare the in vitro motility of RKO cells, and the KLF4 mRNA and protein expression levels of the two cell strains were detected by RT-qPCR. At the same time, the KLF4 mRNA and protein expression levels of the two cell strains were examined by RT-qPCR. RESULTS: The levels of strain KLF4 mRNA and protein expression of RKO cells in the experimental group were lower than those in the control group after dagliflozin intervention, and the differences were statistically significant (P<0.05). And the in vitro motility of strain RKO cells in the experimental group was significantly lower than that of the control group, and the difference was statistically significant (P<0.05). Conclusion: Dagliflozin can play an inhibitory role on the invasive ability of RKO cells, and also effectively reduce the expression level of KLF4, which can have an important impact on colon cancer occurrence and progression by regulating the KLF4-NDRG2 signaling pathway.

KEYWORDS

Dagliflozin; KLF4-NDRG2 signaling pathway; colon cancer; KLF4 mRNA expression level

1. Introduction

Colon cancer is one of the most common cancers in the world in terms of morbidity and mortality, especially in middle-aged and elderly people over 40 years old, and the incidence rate is higher in men (Qiu et al., 2021; Soerjomataram and Bray, 2021). There are many clinical treatments for colon cancer, but the overall prognosis of patients is poor, therefore, in-depth exploration of the molecular mechanisms of colon cancer can further improve the accuracy of treatment and maintain the safety of patients' lives (O'Sullivan et al., 2022; Hestetun et al., 2021). Kruppe-like factor (KLF4) can play an inducing role in the formation of multipotent stem cells, and also play a positive role in the multidirectional differentiation of embryonic stem cells (O'Sullivan et al., 2022). N-Myc downstream regulator gene 2 (NDRG2) is a candidate gene for cancer inhibition, which is associated with the degree of tumor malignancy as well as the occurrence and development of a variety of tumors, and NDRG2 is associated with tumor stage, prognosis, and survival rate of patients, but the mechanism by which NDRG2 inhibits the proliferation of tumor cells in colon cancer is still unclear at this stage. With the increasing trend of population aging in China, diabetes mellitus has become an important chronic metabolic disease that damages public health, and improper control easily induces eye disease, cardiovascular disease and other complications, and studies (Ying et al., 2022) have shown that diabetes mellitus and colon cancer are correlated, and that high-calorie diets, lack of physical activity and obesity are important risk factors for diabetes mellitus and colon cancer. Dagliflozin is a new type of drug for the treatment of diabetes mellitus, and study (Saito et al., 2015) showed that dagliflozin can cause the death of human colon cancer cell HCT116, which can play a certain role in inhibiting colon cancer. The present study investigates the role of dagliflozin in regulating the KLF4-NDRG2 model pathway in inhibiting colon cancer development and progression, the study period was from July 2023 to October 2023, the experimental process as well as the results of the study are reported as

follows.

2. Materials and Methods

2.1 Instrumentation and material reagents

Instruments and equipment include centrifuge (model: AG22331, can be ultracentrifuged at low temperature), pipette, refrigerator (-80℃ ultra-low temperature), DSLR camera, microscope (fluorescence optics), chemiluminescence imaging system (model: ChimiDocTMXRS+), electrophoresis instrument (model: BG-Power600i), spectrophotometer (ND-2000), CO2 Cell Incubator, Electrophoresis System, Airteh Ultra Clean Bench, Electronic Analytical Balance (Model: ER-120A), Autoclave Sterilizer, Constant Temperature Metal Bath, Enzyme Labeling Instrument, Micro Pipette.

The materials and reagents were as follows: dagliflozin (Manufacturer: AstraZeneca Pharmaceuticals LP, Approval No: Registration No.H20170117, Specification: 5mg*14 tablets), Elisa kit (purchased from Shanghai Enzymology Co., Ltd.), Full-scale gold fluorescence quantification kit (purchased from Acres Biologicals), RPMI 1640 medium, EMEM F12 medium, PBS powder, serum-free cell freezing solution, cell culture well plates, protein lysate, antibody dilution solution, RIPA protease inhibitor. Cell lines: human colon cancer cell lines SW620, RKO, SW480.

2.2 Experimental methods

2.2.1 Dagliflozin solution preparation

Dagliflozin solution is prepared as follows: Dagliflozin powder 10mg was prepared into a storage solution (10mm), ensuring that blowing was sufficient to fully dissolve the powder, and then dispensing the solution with a volume of 5μL per tube, and storing it properly at -20℃. At the time of application, dilute the storage solution with complete culture medium such as F12 and DMEM until a certain number of times, and prepare a solution with a concentration of 2 μm. The cell culture method is as follows: human colon cancer cell line should be cultured in 10% fetal bovine serum complete medium, and the cell culture incubator (5% CO2, temperature: 37.5℃) should be used for culture, and the frequency of changing the medium should be 1 time/1-2 d or according to the growth status of the cells. The PBS buffer should be kept at the pH value of 7.2-7.4, and the PBS buffer should be placed into an autoclave for 60 min continuously. The PBS buffer should be placed in an autoclave for 60 min, and then stored in a refrigerator at 4℃ after natural cooling, and can be used the next day.

Cell resuscitation was performed as follows: open the water bath and preheat the water bath at 37℃, place a cell freezing tube with frozen cells into the 37℃ water bath, shake rapidly until completely melted, then centrifuge (speed: 1000r, time: 3min), then add 10% fetal bovine serum 1mL of complete medium resuspension, transfer the cell suspension to a cell culture flask, and then add 3-4mL of medium to which was placed in the incubator for continuous cultivation, and the liquid could be changed after the cells were attached to the wall.

2.2.2 Effect of dagliflozin on RKO cell migration

Ten RKO cell lines were divided into control and experimental groups. The control cells were spread into 6-well plates (2×106 cells/well) with RKO cells (treated with 2 μm DMSO for 48 h), while the experimental cells were pre-stimulated with 2 μm dagliflozin, and the cells were pipetted with a pipette tip (10 μL) through the center of the circle to make a line (in the form of a cross) after attachment to the wall, and the cells were washed three times with PBS after successful operation to Remove the detached cells. Three mL of medium containing 1% PFB was added to each well of the plate, and five observation fields were taken and photographed. The cells were then incubated in an incubator at a frequency of 1 time/12h, and the same field of view was taken to observe the improvement of the delineation, and the cell migration distance was calculated at the same time.

2.2.3 Effect of dagliflozin on KLF4 mRNA and protein expression levels in RKO cells

(1) RKO cells were spread in 6-well plates, dagliflozin was added when the cell density grew to 90%, DMSO was used as a negative control, and the cells were collected after 24 h. Total RNA was extracted and cDNA was obtained using a reverse transcription kit, followed by RT-qPCR using the gene primers of KLF4, with 5 replicates set up for each sample.

(2) RKO cells were spread in 6-well plates, when the cell density grew to 90%, dagliflozin was added and DMSO was used as a negative control, the cells were collected after 24 h. The cells were lysed using RAPI, and the KLF4 protein content within the cell lysates was detected using the Elisa kit for KLF4, and 5 replicated experimental wells were set up for each group.

2.2.4 Statistical analysis

SPSS 26.0 software was used to analyze the data. Normally distributed measurements were expressed as mean ± standard deviation ($\bar{x}$ ± s), and comparisons between groups were made using the independent samples t-test; counting data were expressed as the number of cases (n), and the percentage (%) descriptions were expressed using the χ2 test. p<0.05 was taken as the difference was statistically significant.

2.3 Observation indicators

(1) Comparison of RKO cell motility in vitro.

(2) Compare KLF4 mRNA and protein expression levels by RT-qPCR.

3. Results

3.1 Comparison of RKO cell motility in vitro

The in vitro motility of RKO cells of the experimental group cell strain

Table 1: Comparison of RKO cell motility in vitro ($\bar{x}$ ± s)

Clusters	12 h	48 h
control group(n=5)	42.07±2.31	75.36±2.36
experimental group(n=5)	24.25±2.39	40.26±2.21
T	11.988	24.275
P	<0.001	<0.001

Table 2: Comparison of KLF4 mRNA and protein expression levels ($\bar{x}$ ± s)

Clusters	KLF4 mRNA	KLF4 protein
control group(n=5)	1.01±0.11	0.48±0.07
Experimental group(n=5)	0.35±0.02	0.14±0.03
T	13.200	9.983
P	<0.001	<0.001

was significantly lower than that of the control group, and the difference was statistically significant (P<0.05). Refer to Table 1.

3.2 Comparison of KLF4 mRNA and protein expression levels

The KLF4 mRNA and protein expression levels of the cell strains in the experimental group were lower than those in the control group, and the differences were statistically significant (P<0.05). Refer to Table 2.

4. Discussion

Colon cancer is a highly prevalent malignant tumour worldwide, and the incidence and mortality rates of colon cancer in China are high, respectively 15/100,000-25/100,000 agents 7/100,000-13/100,000, and the burden of colon cancer has increased. At this stage, the clinical pathogenesis of colon cancer cannot be fully elucidated, but oncogenes and cancer suppressor genes play a crucial role in the process of colon cancer development and progression (Chunxiao et al., 2020; Narayanankutty, 2019). With the increasing number of ways and means of clinical treatment of colon cancer, effective biomarkers are important for accurately predicting the development of colon cancer tumours as well as evaluating the prognosis of patients.

Diabetes mellitus patients have lower sensitivity to insulin, which can easily cause insulin resistance, and insulin in the body can not be effectively used, and then hyperinsulinemia can be formed, and hyperinsulinemia is an important factor to promote the occurrence and progression of colon cancer. Colon cancer cells use glucose as the energy source and aerobic glycolysis as the glucose metabolism mode, which is less efficient, so the affinity of tumour cells for glucose increases significantly, and the chronic hyperglycemia of diabetic patients provides favourable conditions for the proliferation of tumour cells and disease progression (Hu, 2020; Xuan et al., 2018). Dagliflozin is a highly effective SGLT2 inhibitor, which is widely used in the treatment of type 2 diabetes mellitus, with ideal glucose-lowering effect, and also has the effect of protecting the kidney and heart, with high patient tolerance, and can act independently of insulin, and does not cause the risk of increased risk of intensive insulin therapy (Hu, 2020., Huang et al., 2021).

As a key factor for inducing the formation of multipotent hepatocytes, KLF4 plays an important role in maintaining the self-renewal of embryonic stem cells, etc. Overexpression of KLF4 can increase the proportion of tumour stem cells, which suggests that KLF4 has an oncogenic effect, and the results of Zhao et al.'s study (Zhao et al., 2020) showed that KLF4 overexpression existed in colon cancer patients and was used as a marker for the early invasiveness of the phenotype. KLF4 is a transcription factor that can slow down apoptosis and senescence by inhibiting p53 transcription, which may be related to the decrease in apoptosis rate of tumour cells caused by KLF4 overexpression. NDRG2 belongs to the NDRG gene family, which is localized on chromosome 14q11.2, and it exists in a wide range of organs such as kidneys, brain, liver, heart as well as small intestines, colon, and so on, and can play a role in the growth, proliferation, and metastasis of colon cancer. It can play a role in the growth, proliferation and metastasis of colon cancer. Abnormal methylation of NDRG2 can lead to the down-regulation of NDRG2 expression, resulting in the inability of its oncogene function to play its normal role, which leads to the abnormal function of tumour cell differentiation, proliferation and apoptosis, etc. NDRG2 can affect the development and progression of tumours by participating in and regulating a variety of signalling pathways. As an oncogene, NDRG2 plays a role in the development and progression of many types of tumours, including malignant tumours of the digestive tract, such as colon cancer, etc. A large number of studies have demonstrated that NDRG2 participates in tumour progression through a variety of mechanisms, such as silencing of the methylation of the NDRG2 promoter and the interaction with LncRNAs,etc., and also involves cell junction-associated proteins, molecular targets and signalling pathways. pathways, etc.

The KLF4-NDRG2 signalling pathway belongs to the important regulatory metastatic factors in cancer, which is frequently activated in human tumours and can be used as a therapeutic target with important applications. KLF4 signalling, when activated, can promote tumour proliferation by way of regulation of downstream cell cycle regulators, while NDRG2 function is directly related to colon tumour cell proliferation and survival (Zhao et al., 2023). In this study, the KLF4 mRNA and protein expression levels of the experimental group strains were lower than those of the control group, and the in vitro motility of the experimental group strain RKO cells was significantly lower than that of the control group (P<0.05). By comparison, it can be seen that dagliflozin can effectively inhibit the invasive ability of RKO cells, and also can have an inhibitory effect on the expression level of KLF4, which can have an effect on colon cancer occurrence and progression by way of regulating the KLF4-NDRG2 signalling pathway. In summary, dagliflozin can effectively inhibit the invasion ability of RKO cells and reduce the expression level of KLF4. Dagliflozin can affect the occurrence and progression of colon cancer by regulating the KLF4-NDRG2 signalling pathway, which provides a reference guide for the clinical selection of reasonable drugs for the treatment and control of colon cancer, however, its cancer inhibitory effect in colon cancer and its safety still need to be verified by a large number of researches and experiments, so as to provide a basis for controlling the condition of colon cancer patients and improving their prognosis. However, its cancer inhibitory effect and safety in colon cancer still need to be verified by a large number of studies and experiments, so as to provide a basis for controlling the condition of colon cancer patients and improving their prognosis.

Conflicts of Interest

To authors declare that they have no conficts of interest.

Acknowledgments

This study was supported by the project of Innovation and Entrepreneurship Training Programme for College Students (x202310742286) and the project of Lanzhou Municipal Bureau of Science and Technology, "Exploring the effect of Tianwang Tonicardin on cognitive dysfunction after heart failure based on the theory of reverse molecular docking and cerebral-cardiac homoeopathy" (2023-ZD-4).

References

Chunxiao, W., Kai, G., Yangming, G., et al. 2020. Analysis of colorectal cancer incidence and death in China in 2015. Chinese Journal of Cancer, 30(4), Pp. 241-245.

Hestetun, K. E., Aasebo, K., Rosenlund, N.B., et al. 2021. Mismactch repair phenotype determines the implications of tumor grade and CDX2 expression in stage II-IIIcolon cancer. Mod Pathol, 34(1), Pp. 161-170.

Hu, W. 2020. Mechanism study of dagliflozin inhibiting colorectal cancer metastasis through PI3K/AKT/mTOR signalling pathway. Southern Medical University.

Hu, W. 2020. Multiplex immunohistochemistry/immunofluoresecence is superior to tumor mutational burden and PD-L1 immunohistochemistry for predicting response to anti-PD-1-L1 immunotherapy. Thorac Cancer, 11(1), Pp. 13-5.

Huang, W.F., Chen, Q.H., Xue, H.L., et al. 2021. Meta-analysis of the correlation between dagliflozin and the risk of malignant tumour. Journal of Adverse Drug Reactions, 23(6), Pp. 304-311.

Narayanankutty, A. 2019. PI3K/Akt/mTOR pathway as a therapeutic target for colorectal cancer:A review of preclinical evidence. Current Drug Targets, 4(20), Pp. 1217-1226.

O'Sullivan, D.E., Sutherland, R.L., Town, S., et al. 2022. Risk factors for elderly-onset colorectal cancer: a systematic review and meta-analysis. Clin Gastroenterol Hepatol, 20(6), Pp. 1229-1240.

Qiu, H., Cao, S., Xu, R. 2021. Cancer incidence, mortality, and burden in China: a time-trend analysis and comparison with the United States and United Kingdom based on the global epidemiological data released in 2020. Cancer Commun(Lond), 41(10), Pp. 1037-1048.

Saito, T., Okada, S., Yamada, E., et al. 2015. Effect of dapagliflozin on colon cancer cell(Rapid Communication)[J].Endocrine Journal, 62, Pp. 1133-7.

Soerjomataram, I., Bray, F. 2021. Planning for tomorrow: global cancer incidence and the role of prevention 2020-2070. Nat Rev Clin Oncol,

18(10), Pp. 663-672.

Xuan, Q.F., Cai, L.L., et al. 2018. Expression and clinical significance of NDRG1 and NDRG2 genes in colorectal cancer tissues. Journal of Inner Mongolia Medical University, 40(6), Pp. 554-559,565.

Ying, N.C., Li, G.S., Lei, L., et al. 2022. Relationship between blood glucose level and soluble intercellular adhesion molecule-1 and C-reactive protein in patients with colon cancer combined with diabetes mellitus and the influencing factors of postoperative infection. Cancer Progress, 20(1), Pp. 38-41.

Zhao, J., Zhu, X.L., Jin, X., et al. 2023. MiR-152-3p targets KLF4 to promote colon cancer cell proliferation, migration and invasion. Journal of Wenzhou Medical University, 53(12), Pp. 954-962,968.

Zhao, R., Liu, Z., Xu, W., et al. 2020. Helicopter pylori infection leads to KLF4 inactivation in gastric cancer through a TET1-mediated DNA methylation mechanism. Cancer Med, 9(7), Pp. 2251-2563.