A review of the research on the mechanism of EGCG targeting Foxo3 to inhibit the expression of Bnip3 and LC3 and alleviate autophagy to inhibit colon cancer

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ABSTRACT
Colon cancer is a common malignant tumor, and its pathogenesis is complex and changeable. Studies have shown the potential therapeutic effect of epigallocatechin gallate (EGCG) on colon cancer, especially its inhibition of Bnip3 and LC3 expression by targeting the Foxo3 transcription factor, thereby mitigating the effect of autophagy inhibition on colon cancer cells. Autophagy is aberrantly activated in colon cancer and is associated with tumor growth and drug resistance. As a green tea extract with antitumor, antioxidant and anti-inflammatory functions, EGCG can regulate the expression of autophagy-related proteins and affect the growth and metastasis of cancer cells. Studies at home and abroad have shown that EGCG regulates autophagy by inhibiting Foxo3 activity, and has potential anti-tumor activity against colon cancer. However, the mechanism of action of EGCG in vivo is not well understood, and future studies need to further explore its role in animal models and clinical samples, as well as its effects on tumor cell metabolism, apoptosis, proliferation and metastasis, in order to provide a new strategy for the treatment of colon cancer. The purpose of this article is to provide systematic theoretical support for EGCG targeting Foxo3 to inhibit the expression of Bnip3 and LC3 and alleviate autophagy to inhibit colon cancer。

Keywords: Colon Cancer; EGCG; research progress; Review

Autophagy is an important survival mechanism for cells under nutrient deprivation or other stressful conditions, and it maintains the stability of the intracellular environment by degrading intracellular proteins and organelles. However, aberrantly activated autophagy has also been implicated in the development of a variety of diseases, including tumors. In malignancies such as colon cancer, inhibition of autophagy is often considered a key factor in promoting tumor growth and drug resistance. Therefore, the search for molecular targets that can regulate autophagy and have therapeutic potential for tumor cells has become one of the hot topics in the field of cancer research.

EGCG is a natural compound extracted from green tea that has been extensively studied for its various biological functions such as anticancer, antioxidant and anti-inflammatory. Recent studies have shown that EGCG can exert its anti-tumor effects by regulating the expression of autophagy-related proteins. Foxo3 is an important transcription factor that plays a key role in regulating processes such as autophagy and apoptosis. Bnip3 and LC3 are key proteins in autophagy.

1. Background
Autophagy is an important metabolic process in cells, which plays an important role in maintaining the stability of the intracellular environment and coping with various stresses. However, the autophagic process in cancer cells is often inhibited, which promotes tumor growth and metastasis. Therefore, it is of great significance to study how to inhibit the growth and metastasis of cancer cells by regulating autophagy.

EGCG is a natural catechin compound that has been shown to have a variety of biological activities such as antioxidant, anti-inflammatory, and anti-tumor. Recent studies have shown that EGCG can regulate autophagy by targeting Foxo3, which in turn affects the growth and metastasis of cancer cells. Foxo3 is one of the key regulators of autophagy, and it plays an important role in the process of autophagy.

Studies have also found that Bnip3 and LC3 are autophagy-related proteins, and their abnormal expression in tumors is closely related to the occurrence and development of tumors.

2. Review of foreign literature

EGCG targets Foxo3 to inhibit the expression of Bnip3 and LC3, and alleviate the mechanism of autophagy and inhibition of colon cancer

Autophagy is an important biological process in which cells self-degrade and regenerate, and has been shown to play a key role in many diseases, including cancer. In colon cancer, aberrant activation of autophagy is closely related to tumor growth and metastasis.

In recent years, researchers have discovered that EGCG (catechin-3-O-gluconate) has potential anti-tumor activity as a natural polyphenolic compound. EGCG regulates the biological behavior of cancer cells through a variety of pathways, including influencing the process of autophagy. It was found that EGCG can modulate autophagy by inhibiting the activity of Foxo3 (franklin-rich protein kinase 3), which provides a new idea for further research on the application of EGCG in the treatment of colon cancer.

Foxo3 is an important transcription factor that plays an important role in maintaining cellular homeostasis and responding to cellular stress conditions. Recent studies have shown that Foxo3 also plays a key role in the regulation of autophagy. Activation of Foxo3 can promote the process of autophagy, while its inhibition inhibits autophagy. In addition, Foxo3 can also affect autophagy by regulating the expression of Bnip3 (Bcl-2 interacting protein 3) and LC3 (autophagosome-associated protein 8). Bnip3 and LC3 are two key proteins in the autophagy process, and their expression levels are closely related to autophagic activity.

A recent study has revealed a novel mechanism of EGCG regulation of autophagy. The study found that EGCG could inhibit the activity of Foxo3, thereby reducing the expression of Bnip3 and LC3. This result suggests that EGCG inhibits the autophagy process by inhibiting Foxo3, providing a new strategy for colon cancer treatment.

In addition, the study also found that the inhibitory effect of EGCG on colon cancer cells is closely related to its autophagy regulation in vivo. The results showed that EGCG could significantly inhibit the growth and metastasis of colon cancer cells, and this inhibitory effect was related to its effect on the regulation of autophagy. This suggests that EGCG may be an effective treatment for colon cancer.

EGCG regulates the expression of Bnip3 and LC3 by inhibiting Foxo3, thereby inhibiting the autophagic process, and has potential antitumor activity. This discovery provides a new idea and method for the development of treatment strategies for colon cancer.

3. Review of domestic literature

Wang Xia (2021) proposed a novel mechanism to explain the process of colon cancer resistance to fluorouracil and cetuximab antigen. It was found that MMP activation led to the induction of the Sdc-1/HB-EGF/EGFR/AUTOPHAGY pathway, thereby promoting the development of drug resistance. Further experiments have shown that EGCG can reverse this process by inhibiting MMP, thereby increasing the sensitivity of drug-resistant cells to fluorouracil and cetuximab. This study provides a new perspective for understanding the mechanisms of drug resistance in colon cancer and provides a theoretical basis for the development of new therapeutic strategies.

Shasha Jin (2023) proposed that EGCG antagonizes its therapeutic effect by inhibiting the Hippo signaling pathway and activating YAP protein activity. It was found that EGCG treatment down-regulated the homeostasis of LATS1/2 protein, activated the activity of the chaperone HSP90 cofactor CHIP, promoted the stabilization, hypophosphorylation, nuclear transport of YAP protein downstream of LATS1/2, and activated the transcription of target genes CTGF, CYR61 and ANKRD1 downstream of YAP protein. However, activated YAP antagonizes the therapeutic effect of EGCG by inducing cancer cells that were originally resusctured to re-enter the cell cycle. Therefore, inhibition of YAP activity in colorectal cancer cells can increase the antitumor effect of EGCG.

Li Jianfei (2022) proposed that the main bioactive component in green tea, gallocatechin gallate (EGCG), inhibits the overactivation of Notch by targeting the Notch signaling pathway, thereby alleviating the effect and molecular mechanism of DSS-induced acute ulcerative colitis. Studies have found that EGCG can significantly reduce the level of inflammatory factors, the rate of apoptosis, restore the intestinal barrier function and other pathways, and play a relieving role.

Du Bingxin (2022) proposed that catechins EGCG and ECG can inhibit the proliferation of melanoma cells, induce apoptosis, and reduce the level of autophagy, which has a certain therapeutic effect on melanoma. Experimental studies have found that EGCG and ECG have obvious inhibitory effects on human melanoma A375 cells, and can induce apoptosis while reducing the level of autophagy. In addition, EGCG can also effectively inhibit the increase of tumor volume and induce apoptosis of tumor tissue in nude mouse malignant melanoma models. This suggests that catechins, EGCG and ECG may be potential drugs for the treatment of melanoma.

Li Yuting (2019) proposed that EGCG can inhibit the growth of bladder cancer cells and promote their apoptosis. In addition, EGCG may induce autophagy in bladder cancer cells by upregulating autophagy-related proteins. The study also found that EGCG may promote autophagy and apoptosis of bladder cancer cells by downregulating the PI3K/AKT/mTOR pathway.

Yang Zhizhen (2022) proposed that EGCG, as the main active ingredient in green tea, has a variety of benefits such as antioxidant and antitumor. It was found that EGCG can act as an inhibitor of hFEN1, specifically affecting DNA replication and repair in tumor cells without affecting normal cells. By constructing MRE11-deficient gastric cancer cell lines and mouse xenograft models, EGCG was determined to have a significant therapeutic effect on MRE11-deficient gastric cancer cell lines. The CRISPR/Cas9 screening system was used to construct human genome-wide knockout library cells, and high-throughput sequencing analysis showed that the MAPK11 gene may be related to the inhibition of EGCG. Finally, by inhibiting the expression of MAPK11 kinase, EGCG was verified to exert anti-tumor effects by targeting hFEN1 to achieve synthetic lethal effects. This study provides a scientific basis for the development of new targeted anti-tumor therapy methods and anti-cancer pathways for adjuvant cancer treatment.

Liu Zhengguo (2019) proposed that EGCG has a significant inhibitory effect on human thyroid cancer cells, and this effect is dose-dependent. MTT and EdU experiments have found that EGCG can reduce the viability and proliferation rate of thyroid cancer cells. At the same time, the TUNEL assay showed that EGCG could increase the rate of apoptosis. In addition, the results of cell scratching, migration and invasion experiments showed that the wound healing rate, migration and invasion ability of cells decreased after EGCG treatment. The results of HE staining and immunohistochemistry assay showed that the tissue structure became shallow, the cell arrangement was loose, and there were obvious tissue vacancies and apoptotic areas after EGCG treatment. Western blot assay results showed that EGCG could increase the expression of apoptosis-related proteins and exert anti-thyroid cancer effects by inhibiting the EGFR-RAS-RAF-MEK-ERK pathway.

Ge Sitang, Zhang Xiaofeng, He Yifan, Wu Huatao, Zhang Zining, Li Jing, Geng Zhijun, Song Xue, Zuo Lugen (2022) proposed that EGCG can protect TNBS-induced Crohn's disease-like intestinal inflammation, which may be related to the protection of intestinal barrier function and the inhibition of JAK2/STAT3 inflammatory signaling.

Bai Lu (2021) showed that gallocatechin gallate (EGCG) can effectively inhibit the proliferation of prostate cancer PC-3 cells, and may play a role by up-regulating the expression level of P27kip1 and intranuclear expression and down-regulating the PI3K/AKT protein pathway. In addition, EGCG can also inhibit the S phase of the PC-3 cell cycle, thereby inhibiting the growth of prostate cancer cells.

Tian Chunyang, Zhang Meng, Xia Yongxin, and Zhang Xiangdong (2022) found that theaflavins (TF) can inhibit the proliferation of colon cancer cells and promote their apoptosis by upregulating the expression of circular RNA fork head box protein 3 (circ-Foxo3). The results showed that the cell survival rate was significantly reduced, the apoptosis rate was significantly increased, and the expression of circ-Foxo3 also increased. In addition, transfection of pcDNA-circ-Foxo3 could further verify that TF played a role by up-regulating circ-Foxo3, while transfection of si-circ-Foxo3 could inhibit the effect of TF on the proliferation and apoptosis of HCT-8 cells. These findings provide a novel mechanistic explanation for theaflavins as a potential anticancer drug.

Cai Biqing (2022) explored the expression level of nuclear factor E2-related factor 3 (Nrf3) in colorectal cancer and its effect on cellular antioxidant effects. The study found that the transcription level and protein expression level of Nrf3 in colorectal cancer tissues were significantly increased. Experiments have found that colorectal cancer cell lines overexpressing Nrf3 can reduce H_2O_2-induced oxidative stress damage, and this protective effect may be related to P-AKT, Bcl-2, P-JNK, and P-p38 proteins. These results suggest that Nrf3 has an important regulatory role in colorectal cancer.

Zuo Gaolong (2020) found that epigallocatechin gallate (EGCG) in tea can effectively reduce body weight, body fat percentage, and blood lipid levels in rats with nonalcoholic fatty liver disease (NAFLD), and improve glucose metabolism and insulin resistance. In addition, EGCG can also alleviate liver lipid aggregation in NAFLD rats, reduce the expression of liver TLR4/MYD88/NF-κB signaling pathway proteins and pro-inflammatory cytokines, and reduce liver oxidative stress by virtue of its strong antioxidant capacity. At the same time, EGCG also plays an important role in the maintenance of intestinal homeostasis, which can reduce the levels of systemic endotoxin and diamine oxidase, increase intestinal V/C value, protect the integrity of the intestinal mucosal barrier, change the composition of intestinal microbes, and increase the relative abundance of short-chain fatty acid-producing bacteria. This study provides a scientific basis for exploring the role and mechanism of EGCG in improving NAFLD and related metabolic diseases.

Yanhua Sun, Ming Li, Jianli Xu, Lu Dong, Xuebin Cao, Xiaojing Meng (2020) found that shikonin can induce apoptosis and autophagy in human colon cancer cell line SNU-407 cells in a time- and dose-dependent manner. Specifically, autophagy-related E1 ligase 7 (ATG7) expression levels were significantly increased after shikonin treatment, while silencing of ATG7 inhibited shikonin-induced autophagy. In addition, shikonin also regulates the expression of death receptor (Caspase-8, Caspase-3, PARP) protein by activating the mitogen-activated protein kinase (MAPK) signaling pathway and promotes apoptosis. These results suggest that shikonin may activate apoptosis and autophagy through the ATG7 signaling pathway, which has a potential therapeutic effect on human colon cancer cells.

Huo Miaomiao (2020) proposed the key role of the IRE1α-XBP1s signaling pathway in chemoresistance of

colorectal cancer. The study found that the activation status of EGFR and IRE1α in colorectal cancer patient specimens was abnormally elevated, which was associated with the high expression of EGFR. Further studies have shown that EGFR activation can promote the phosphorylation level of IRE1α and the cleavage of XBP1s, while inhibition of EGFR down-regulates the IRE1α-XBP1s signaling pathway. In addition, there is an interaction between ERK and IRE1α, and inhibition of ERK activity can reverse the activation of IRE1α-XBP1s signaling caused by EGFR activation. The study also found that the chemotherapy drug oxaliplatin can induce the activation of IRE1α-XBP1s signaling in tumor cells, while the monoclonal antibody cetuxib targeting EGFR can inhibit this phenomenon. Therefore, the chemotherapy drug oxaliplatin combined with cetuximab targeting EGFR can improve its therapeutic effect by reducing chemotherapy resistance, which provides a certain reference for the clinical treatment of colorectal cancer.

Wang Zhe (2020) proposed that FOXP3 is highly expressed in colorectal cancer and liver metastases, and plays a role in promoting the proliferation, invasion and migration of colorectal cancer cells. The results showed that the expression of FOXP3 was associated with the stage of bowel cancer, the occurrence of simultaneous liver metastasis, KRAS mutations and MSI-H status. FOXP3 is involved in regulating the occurrence of liver metastasis in colorectal cancer by promoting the entry of β-catenin into the nucleus and directly binding to TCF4 to form a transcriptional costimulatory factor, and activating the Wnt/β-catenin signaling pathway. In addition, FOXP3 also promotes the expression of MMP9 by affecting the SAM metabolism of methionine cycle, which further promotes the occurrence of liver metastasis in colorectal cancer.

Vladislav Snitsarev, Elena Petroff, David P. Rotella (2021) showed that the fluorescence intensity of apparent green tea polyphenols (EGCG) was negatively correlated with the spontaneous dissociation constant (Kap) of the solvent. This means that the fluorescence intensity of EGCG is lower in a protonative environment with a higher Kap value, and higher in a non-protonative environment with a lower Kap value. In addition, the addition of HCl decreases the fluorescence intensity of EGCG, while the addition of NaOH increases its fluorescence intensity. The study also found that the addition of NaOH caused a slow and brief change in EGCG fluorescence intensity over about 10 minutes. These findings reveal the relationship between EGCG fluorescence and environmental protonability, providing useful information for exploring the specific interactions of EGCG in protonative protein binding sites.

Wei Ma, Christophe Tribet, Sylvain Guyot, Dražen Zanchi (2019) investigated the effect of green tea polyphenol epifunin (EGCG) on the formation of β-amyloid aggregates induced by heat stress by dynamic light scattering and small-angle X-ray scattering. It was found that two aggregates, microclusters and fibers, dominate the evolution of light scattering intensity and effective hydrodynamic diameter over time. SAXS experiments were able to distinguish microclusters and fibers, so the variation of the scattering profile over time revealed the structural evolution of the two populations. The low-Q scattering intensity decreases rapidly before the expected increase due to fiber growth, a phenomenon that is interpreted as the release of monomers in the microclumps. This suggests that under thermal stress, free native monomers are converted into monomers that are prone to the formation of β-amyloid, resulting in the release of natural monomers from microblocs. The study also found that EGCG does not bind to protein fibers and does not affect or prevent the addition of monomers to growing fibers. Based on these facts, the authors propose a kinetic model of EGCG-controlled microblob protein β-amyloid aggregation, in which the growth rate inhibition function is introduced to quantify the effect of EGCG on fiber growth.

Minghan Chen, Chunrui Xu, Ziang Xu, Wei He, Haorui Zhang, Jing Su, Qianqian Song (2022) proposed a method that combines bioinformatics and systems biology for comprehensively unraveling the function and signaling pathways of lung cancer therapeutics. By analyzing time-series data from lung adenocarcinoma-derived A549 cells after DEX treatment, they first identified differentially expressed genes and, through queries of regulatory networks, identified key hub genes, including TGF-β, MYC, and SMAD3. Further enrichment analysis revealed that the TGF-β signaling pathway was the most enrichment term. These genes involved in the TGF-β pathway and their cross-interaction with the ERBB pathway have shown a strong survival prognosis in clinical lung cancer samples. In addition, based on biological validation and further curation, they developed a multiscale model of tumor regulation centered on TGF-β-induced and ERBB-amplified signaling pathways to describe the dynamic effects of DEX treatment on lung cancer cells. Their simulation results agree well with the available data for SMAD2, FOXO3, TGFβ1 and TGFβR1 on the time series. In addition, they provide predictions for different doses, demonstrating trends and therapeutic potential for DEX treatments.

Tavassoly, J. Parmar, A. N. Shajahan-Haq, R. Clarke, W. T. Baumann, J. J. Tyson (2013) proposed a mathematical model for analyzing the decision-making process from autophagy to apoptosis in response to mild and severe stress. This model is based on quantitative measurements of autophagy and apoptosis in rat adrenal cortex cells under Staphylococcus aureus-induced stress. By building this model, it is possible to better understand the aberrant regulatory mechanisms of autophagy and apoptosis in disease, especially in cancer.

Goutam Kumar Chandra, Debi Ranjan Tripathy, Swagata Dasgupta, Anushree Roy (2010) investigated the interaction of silver nanoparticles with (-)-epicatechin gallic acid (EGCG). Surface plasmon resonance

experiments showed that EGCG molecules removed borate ions from the surface of silver particles through their coordination properties. In addition, NMR studies and pH titration further confirmed the complex formation of EGCG with borate ions. The researchers have proposed a possible mechanism for the interaction between the two.

4. Research conclusions at home and abroad

EGCG, as a natural polyphenolic compound, has potential antitumor activity in colon cancer treatment. Recent studies have shown that EGCG can regulate the autophagy process by inhibiting the activity of Foxo3, thereby affecting the expression of Bnip3 and LC3. However, there are still some shortcomings and limitations in the current research on the mechanism of EGCG regulation of autophagy.

First of all, the existing studies have mainly focused on in vitro cell experiments, and the effects of EGCG on autophagy regulation in vivo are poorly understood. Therefore, future studies need to further explore the mechanism of action of EGCG in animal models or clinical samples to verify its regulatory effect on autophagy in vivo.

Second, although EGCG has been found to inhibit the activity of Foxo3, its specific mechanism of action is still unclear. Future studies need to further reveal how EGCG regulates the activity of Foxo3 by affecting its phosphorylation, ubiquitination and other signaling pathways, and further study its effects on the expression of Bnip3 and LC3.

In addition, existing studies have mainly focused on the effect of EGCG on the regulation of autophagy, while other possible anti-tumor mechanisms have not been fully studied. Therefore, future studies can further explore the effects of EGCG on the biological behaviors of tumor cell metabolism, apoptosis, proliferation, and metastasis, as well as the relationship between these effects and its autophagy regulation.

In conclusion, EGCG, as a potential antitumor drug, is of great significance in regulating the autophagy process. However, there are still many unknowns about its mechanism of action and potential for clinical applications. Therefore, future studies need to further explore the mechanism of action of EGCG in the treatment of colon cancer, and conduct relevant preclinical and clinical studies, in order to provide a scientific basis for the development of new treatment strategies.

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