REVIEW
Traditional Medicine Research 2023;8(3):18. https://doi.org/10.53388/TMR20220901001

Baicalin: a prominent therapeutic agent against colorectal cancer

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Author contributions

Yin-Zi Yue and Shuai Yan designed and wrote the main content of manuscript. Jin Xie revised the figures and tables, respectively. Yin-Zi Yue and Shuai Yan validated that the descriptions are accurate and agreed upon by all authors.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments

This work was funded by the Fifth Batch of Gusu Health Personnel Training Project in Suzhou (GSWS2020085), Natural Science Foundation of Nanjing University of Chinese Medicine (XZR2020038), Suzhou Science and Technology Development Plan (SYSD2019213), Science and Technology Innovation Project of Suzhou Medical and Health Care (SKJY2021136). We thank American Journal Experts from Research Square Company for editing the English text of a draft of this manuscript.

Peer review information

Traditional Medicine Research thanks Long Ma, Sadique Hussain and other anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations

CRC, colorectal cancer; CDK, cyclin-dependent kinase; ROS, reactive oxygen species; DKK1, Dickkopf1; SP1, specific protein 1; EMT, epithelial-mesenchymal transition.

Citation

Yue YZ, Xie J, Yan S. Baicalin: a prominent therapeutic agent against colorectal cancer. *Tradit Med Res.* 2023;8(3):18. doi: 10.53388/TMR20220901001.

Executive editor: Guang-Ze Ma.

Received: 01 September 2022; **Accepted:** 10 October 2022; **Available online:** 17 November 2022.

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Abstract

The occurrence and development of colorectal cancer involve multiple genes and pathways as a result of accumulated mutations at several sites that control growth and differentiation. Baicalin is a flavonoid extracted from *Scutellaria baicalensis* Georgi with antioxidant, anti-inflammatory and antiviral activities. Here, we discuss its possible clinical development and perspectives for future research. We also summarize the literature on colorectal cancer as well as the anticancer effect of *Scutellaria baicalensis* Georgi in the last 20 years. As research progresses, the therapeutic effect of baicalin in combating colorectal cancer has gradually been recognized. Its impact on colorectal cancer and anticancer mechanism, such as blocking the tumor cell cycle, inducing tumor cell apoptosis, preventing and treating tumor metastasis and anti-inflammatory activity, have become new hot topics in the study of antitumor agents in Chinese medicine. The present review surveys and summarizes studies of baicalin in anti-colorectal cancer treatment to enhance the understanding of its tumoricidal mechanism and to provide new therapeutic options for colorectal cancer.

Keywords: baicalin; colorectal cancer; apoptosis; metastasis; proliferation; treatment; mechanism

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Highlights

Colorectal cancer is a malignant cancer with an increasing occurrence and a high rate of mortality. Baicalin is a traditional Chinese herb, and it is the major flavonoid component derived from the root of *Scutellaria baicalensis* Georgi. This report presents a holistic overview of baicalin for anti-colorectal cancer.

Medical history of objective

Scutellaria baicalensis Georgi, known as traditional Chinese medicine, has long been used for the treatment of dysentery, threatened abortion, and swollen welling abscess diseases. According to the ancient books, it was originally recorded in *Shen Nong Ben Cao Jing* (Sheng Nong's Herbal Classic) in the Eastern Han Dynasty (AD 25–220 A.D.). It was later recorded in Compendium of Materia Medica written by Shi-Zhen Li (552–1578 C.E.), and Bencao Jing Jizhu written by Hong-Jing Tao (220–450 C.E.), etc. Baicalin has been identified to exhibit various pharmacological activities, such as anticarcinogenic, antioxidant, antibacterial, and antitumor activities, and was proven to be non-toxic and safe for use in animals and humans. This paper systematically summarizes the research progress of the mechanism of baicalin in the treatment of colorectal cancer, and discusses the value, significance and existing problems in order to provide reference for the development and utilization of other traditional medicinal plants.

Background

According to the 2018 global cancer statistics, colorectal cancer (CRC) was the third most common cancer and the third leading cause of cancer death in both men and women in the United States [1]. In China, the morbidity and mortality of CRC ranked 3rd and 5th, respectively, among malignant tumors, and are still on the rise [2]. Every sixth death in the world is due to cancer, making it the second leading cause of death (after cardiovascular diseases). Figure 1 shows the estimated age-standardized incidence rates in 2020 attributed to colorectal cancer according to GLOBOCAN 2020 and International Agency for Research on Cancer. Surgical resection together with chemotherapy is now the main treatment for CRC. However, a standard chemotherapy regimen after surgery cannot completely prevent early cancer recurrence among patients. The 5-year survival rate of patients with early-stage CRC approximately 90%, while the overall survival rate of patients with advanced CRC has not significantly improved, and is only around 15% [3]. The occurrence and development of CRC involve multiple genes and pathways as a result of accumulated mutations at several sites that control growth and differentiation. Therefore, it is of great significance to find effective drugs and provide new, safe, and effective treatments to increase the survival rate and to improve the quality of life of patients with CRC [4]. Chinese medicines have shown advantages in the treatment of CRC by improving patient quality of life, increasing the survival rate, reducing the adverse reactions of radiotherapy and chemotherapy, and preventing tumor recurrence and metastasis [5, 6].

Baicalin – a potent bioactive natural medicine for various human diseases

Scutellaria baicalensis is the dried root of *Scutellaria baicalensis* Georgi that was first recorded in *Shengnong's Classic Materia Medica* (unknow author, 25–220 C.E.). It has a variety of curative effects and a long history of applications in Chinese medicine and modern herbal remedies for at least 2,000 years.

After excavation mainly in the spring and autumn, its above-ground part as well as sediment is removed, and the rooty parts are then dried for medical use [7]. *Scutellaria baicalensis* is cold in nature and bitter in taste, attributed to the lung, gallbladder, spleen, large intestine and small intestine meridians. It has been used to clear heat and dry dampness, cool blood and prevent miscarriage, discharge fire and remove toxin in traditional Chinese medicine [8]. *Scutellaria baicalensis* also shows good antibacterial effects in clinical practiceand is thus mainly used to treat lung heat cough, upper respiratory tract infection, dysentery, threatened abortion, swollen welling abscess and clove sores [8]. At present, *Scutellaria baicalensis* has been officially included in the *Chinese Pharmacopoeia* (2015), *European Pharmacopoeia* (EP 9.0) and *British Pharmacopoeia* (BP 2018).

As the major components of *Scutellaria baicalensis*, flavonoids are associated with the various biological activities including anti-inflammatory, antiviral, antioxidant, anti-allergy and antitumor effects. Baicalin, as the main active flavonoid extracted from *Scutellaria baicalensis* Georgi, plays an important role in clearing free radicals, enhancing apoptosis, blocking calcium channels, and inhibiting aldose reductase.

Baicalin is often used to treat pneumonia, cerebral ischemia, hepatitis, infection and tumors in the clinic because of its protective effect on the immune system, cardio-cerebrovascular system, digestive system and nervous system [9]. Baicalin (Figure 2) is a light-yellow powder with phenolic hydroxyl groups in its structure which has an unsaturation of 11 [10]. Baicalin may be distributed in many plants. The clinical usage of baicalin has attracted great attention due to its few side effects and simplicity and low cost.

Materials and Methods

In this review, we focus on the anticancer effects of baicalin in CRC, including the underlying main molecular mechanisms, in vivo studies and clinical applications. Electronic databases including Web of Science, MEDLINE (via PubMed), SciFinder, Google Scholar, Allied and Alternative Medicine and China Journals Full Text Database (via CNKI, VIP and Wanfang), were extensively searched from inception until March 2021. The terms and keywords for searching included *Scutellaria baicalensis* Georgi, Huangqin, Scutellaria, baicalin, anticancer, neoplasm, colorectal carcinoma, colorectal cancer, carcinogenesis and colon carcinoma. No language restriction was

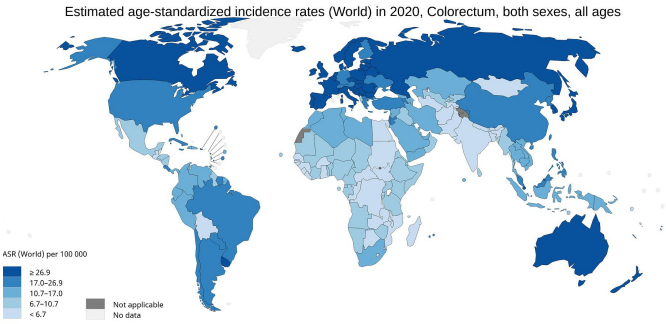

Figure 1 The estimated age-standardized incidence colorectum cancer. This measures colorectum cancer ASR across both sexes and all ages. Smaller categories of cancer types have been grouped by cancer today in data into a collective category “other cancers”. ASR, age-standardised rate.

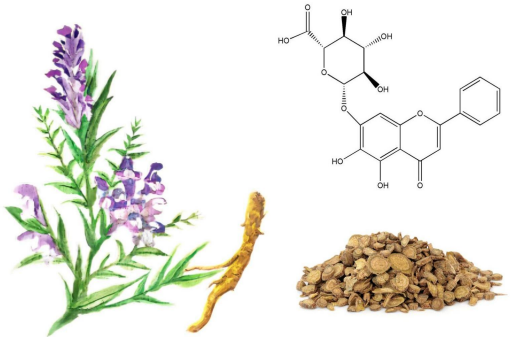

Figure 2 *Scutellaria baicalensis* Georgi and the chemical structure of baicalin. The molecular formula of baicalin is $C_{21}H_{18}O_{11}$.

imposed, but the most relevant studies were published in English and Chinese. The process is summarized in a flow diagram in Figure 3. The underlying molecular mechanisms, pharmacology and anticancer effects of baicalin and its clinical applications in combination with other herbal medications are discussed.

Effect of baicalin on proliferation and apoptosis of CRC cells

Inhibiting the CRC cell cycle. The cell cycle is a process of genetic replication and cell division and is mainly composed of the G0/G1, S, G2 and M phases. However, uncontrolled cell cycle activities, particularly uncontrolled G1/S and G2/M detection points, are closely related to cancer [11]. The restriction point in the G2/M phase is an important stage of cell proliferation. Only cells with complete replication and no damage are able to pass this point and enter the M phase, ensuring an accurate distribution of chromosomes to each daughter cell. The cell cycle is influenced by regulatory genes and a variety of regulatory proteins, such as cyclin and cyclin-dependent kinase (CDK). Baicalin inhibited the growth of orthotopic transplantation tumors of HCT-116 cells in nude mice by blocking tumor cells in the G2/M phase to induce apoptosis [12]. Xu et al. also reported that it could inhibit the growth of orthotopic transplantation tumors of HCT-116 colon cancer cells that lack hm1h1-deficient mismatch repair genes in nude mice [13]. This is probably due to the regulation of mismatch repair genes hm1H1, hMSH2, and PCNA. After being treated with baicalin, the number of colon cancer cells HCT-116, HT-29, and SW480 in G0/G1 phase decreased, with most of the cells stagnated in S phase [14]. Another study demonstrated that baicalin had a significant inhibitory effect on the growth of orthotopic xenografts in nude mice composed of human HCT-116 colorectal cancer cells with the mismatch repair gene hMLH1. Although the precise molecular mechanism of inhibition is still unclear, baicalin might be a potent antitumor drug for CRC with MSI [15].

Cyclin D1 and cyclin B1 are key proteins in the regulation of G1 phase and G2/M phase, respectively. The expression of cyclin D1 and cyclin B1 proteins after baicalin treatment was significantly reduced, suggesting that baicalin blocked the cell cycle in the cyclin pathway and inhibited the proliferation of HCT-116 cells [16]. Further experiments showed that the expression of cyclin D1 in G1 phase was downregulated after baicalin was applied, and both cyclin A and CDK2, the mitogenic factors that promote the transformation of cell division from S phase to G2/M phase, were decreased as baicalin dosage increased, which indicated that baicalin blocked the tumor cell cycle [17]. Finaly, these findings suggested that baicalin affects the cell cycle progression by targeting the cyclins and CDKs in a cell concentration-dependent manner. Figure 4 depicts a schematic representation of the cell cycle regulation and the potential role of baicalin.

Inducing apoptosis of colorectal cancer cells. Baicalin not only regulates the cell cycle but also inhibits tumor growth upregulating proapoptotic genes such as p53 and bax and downregulating antiapoptotic genes such as Bcl-2 and Bcl-6, thereby inhibiting tumor cell proliferation [18]. Feng et al. observed the effect of baicalin on colon cancer SW480 cells treated with different concentrations of baicalin for 48 h [19]. SW480 cells were found to manifest morphological changes of apoptosis, such as cell volume reduction, nuclear fragmentation and chromatin agglutination. The apoptosis rate and damage to the cell membrane increased as the baicalin concentration increased, showing a dose-response relationship.

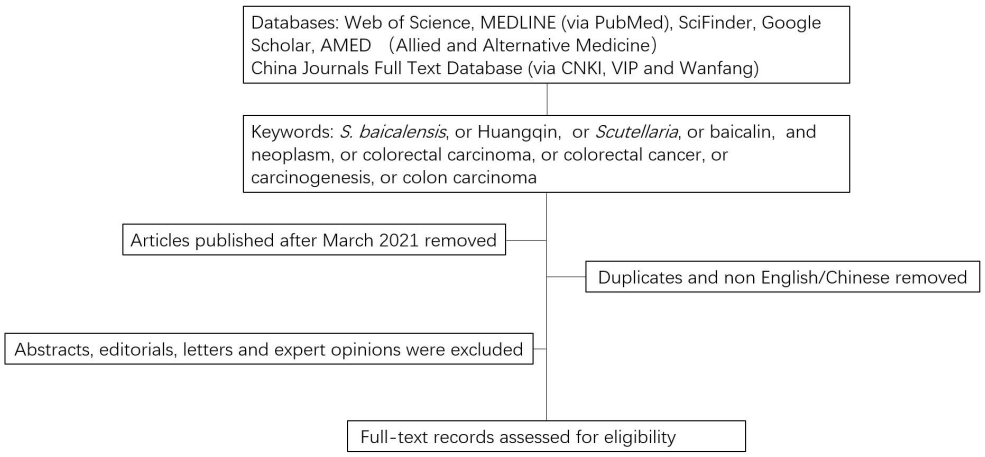

Figure 3 Flow diagram of assessment of identified studies

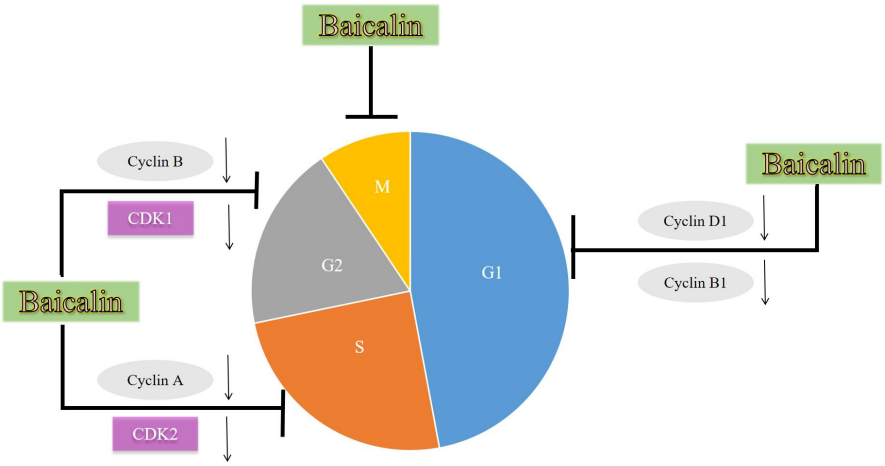

Figure 4 Baicalin regulates the cell proliferation via arresting the G2/M, G1 and S phases of the cell cycle, and down-regulate the Cyclin B, CDK1, Cyclin D1, Cyclin B1 and CDK1. Downside arrows indicate the downregulation of targets/transcription factors. CDK, cyclin-dependent kinase.

Western blot analysis showed a decline in Bcl-2 protein levels and an increase in caspase-3 and caspase-9 expression, which indicated that the apoptosis of SW480 cells induced by baicalin may be related to the reduction in bcl-2 and the mitochondrial pathway.

The caspase recruitment domain with the N-terminus of Apaf-1 recruits the precursor of caspase-9 in the cytoplasm, thereby initiating the caspase cascade reaction. Downstream of caspase-3 is activated, and the corresponding substrate is cleaved to induce apoptosis in SW480 cells [20]. Studies have confirmed that reactive oxygen species (ROS) produced by oxidative stress reactions may act on signaling molecules and regulate cell proliferation, apoptosis, differentiation, autophagy and other cellular activities [21]. Tsai and his team confirmed that baicalin could induce a large number of ROS in SW620 cells to trigger reactions of the caspase family proteins and increase the activities of caspase-3, caspase-8 and caspase-9, ultimately causing the apoptosis of SW620 cells [22]. However, it is currently unknown whether baicalin enhances the activity of NADPH oxidase and xanthine oxidase, or improves the mitochondrial apoptosis pathway, which is worthy of further research.

Survivin is the strongest inhibitor of apoptosis, and plays a significant role in inhibiting apoptosis and cell cycle regulation. It has high tissue distribution specificity and closely related to the pathogenesis and development of cancer [23]. Baicalin effectively promoted the expression of caspase-3, while the expression of survivin decreased significantly in HCT-116 cells. Moreover, survivin expression was found to gradually decline as the baicalin concentration rose. It has been suggested that baicalin induced apoptosis may be attributed to its action of inhibiting survivin expression and enhancing caspase-3 activity [24].

Protein complexes play a main role in mitotic and meiotic chromatin condensation. Each subunit of the condensation protein complex is important for normal separation and condensation of chromatin in mitosis. Therefore, dysfunctional condensation protein complexes affect the normal separation of chromosomes and condensation of chromatin, resulting in chromosome instability, which is a form of genomic instability and a common feature of some cancers. To date, many studies have reported that abnormal function or expression of subunits in condensation protein complex I or II causes cancer [25]. Ncapd2 and ncapd3 belong to subunits of condensation protein complexes I and II, respectively. High expression of ncapd2 and ncapd3 accelerates the cell cycle, inhibits apoptosis, and promotes the growth and proliferation of colon cancer cells [26]. Wei found that baicalin had an inhibitory effect on the growth of HT-29 cells, and this inhibition became more conspicuous as time passed and the baicalin concentration increased [27]. Further studies found that baicalin may inhibit the expression of ncapd2 and ncapd3 to hinder the growth of HT-29 tumor cells and promote the apoptosis and necrosis of tumor cells.

The Wnt signalling pathway is closely linked to the progression of many human tumors, and it plays a variety of important roles in the occurrence and development of colorectal cancer [28]. It has been reported that Dickkopf1 (DKK1) is an important antagonist of the Wnt signalling pathway. It inhibits the classical Wnt pathway in particular by binding up Wnt coreceptor lipoprotein receptor-related protein-6. The induction of DKK1 expression can be used as a target for inactivation of Wnt signal transduction because it can inhibit the classical Wnt pathway by competing to bind the Wnt coreceptor lipoprotein receptor-related protein-6 [29]. It has been shown that baicalin inhibits the Wnt signalling pathway by activating DKK1 in colon cancer cells and upregulates DKK1 expression by downregulating the expression of miR-217 in colon cancer cells to combat cancer [30].

The occurrence of colorectal cancer is a complex and variable process. Abnormal changes in a variety of signalling pathways mediate tumorigenesis, among which the Notch signalling pathway is critical [31, 32]. The Notch signalling cascade is an evolutionarily conserved type I transmembrane receptor protein family that exists widely in vertebrates and invertebrates. Abnormal regulation of the Notch signalling cascade often induces tumors [33]. Notch1 is one of the receptors of the Notch pathway, which is implicated in the development of tumors. In most cases, it is carcinogenic. Previous studies have found that the expression of Notch1 in rectal cancer tissue is significantly higher than that in adjacent normal tissue. Moreover, the abnormal expression of the Notch signalling pathway is directly involved in colon cancer [34]. Jagged1 is one of the important ligands of the Notch pathway, which binds with Notch1 to initiate the Notch pathway and promotes Notch1 to enter the nucleus [35, 36]. Hairy and enhancer of split 1 is one of the important downstream effector genes of the Notch pathway, which can maintain the undifferentiated state of various precursor cells [37]. Liang et al. found that the inhibition rate and apoptosis rate of baicalin on SW480 cells gradually increased with increasing of baicalin dose [38]. Western blotting and reverse transcription polymerase chain reaction were used to investigate the effects of baicalin on the Notch1 and hairy and enhancer of split 1 proteins and the Jagged1 gene in SW480 cells. The results showed a concentration dependent decreasing trend, suggesting that baicalin could inhibit the notch pathway in SW480 cells. Other studies have shown that mouse Notch gene knockout reduced the proliferation of colon cancer cells, promoted apoptosis and reduced colony formation, while overexpression of Notch signalling exhibited the opposite effects [39]. Therefore, we speculate that baicalin can inhibit the proliferation and promote apoptosis of SW480 cells in human colon cancer by negatively regulating the Notch pathway.

Specific protein 1 (SP1), belonging to the SP1/kr ü ppel-like zinc finger protein subfamily, is a transcription factor that is widely present in cells. There are three c2-h2 zinc finger structures at the C-terminus that can interact with target proteins. The GC (GGGCGG) box sequence in the promoter region of the gene is combined to achieve downstream gene transcription [40]. SP1 participates in a variety of inductions related to cell proliferation and survival mechanisms. SP1 siRNA experiments from the past have shown that inhibition of SP1 can induce apoptosis and inhibit the growth of colon cancer stem cells. Therefore, SP1 seems to be the central mediator of colorectal cancer progression [41]. An abnormal increase in SP1 protein levels was observed in patients with this malignant tumor [42]. This study indicated that baicalin induces apoptosis of SW480 cells and downregulates the expression of SP1. In addition, the SP1 inhibitor mithramycin-A induces apoptosis of SW480 cells and reduces the level of SP1 [43]. This mechanism supports the idea of baicalin as a chemoprevention and chemotherapy drug for the treatment of colorectal cancer.

With in-depth studies of the tumor microenvironment, an increasing number of new tumor markers have been identified. miRNAs have a variety of regulatory functions in eukaryotic cells and viruses, which have attracted much attention and have become a hot topic in the field of life science in recent years. Numerous evidences have shown that abnormal miRNA expression directly promotes or suppresses the transcriptional expression of downstream target genes, suggesting that miRNAs are associated with cancer and may have a vital role in diagnosis and prognosis, and may serve as therapeutic targets for CRC [44]. Initial studies of the regulatory effect of baicalin on colorectal cancer cells at the miRNA level showed that baicalin induced and promoted dose-dependent apoptosis of HT-29 cells. It also prevented transplanted tumors in nude mice from growing. We then further performed miRNA microarray analysis of baicaleinz-treated and untreated HT-29 cells found that the expression levels of multiple tumor-inhibiting factors in baicalein-treated HT-29 cells, including miR-10a, miR-23a, miR-30c, miR-31, miR-151a and miR-205, were significantly reduced. Moreover, both in vitro and in vivo studies showed that baicalin lowered the expression level of c-Myc to inhibit tumor growth. In general, this study revealed a new mechanism of baicalin's anticancer effects, that is, by reducing the expression levels of c-myc and oncomiRs baicalin induces apoptosis of colon cancer cells and prevents tumor growth [45]. However, only miR-10a, miR-23a, miR-30c, miR-31, miR-151a and miR-205 were detected in colorectal cancer tissues and cells. Since many miRNAs are involved in the CRC mechanism, it is challenging to explore the effect of baicalin

and its baicalin on the above genes and proteins in the development of CRC. The expression and regulation of these genes in tumor tissues remain to be further investigated experimentally.

Taken together, we summarized the mechanisms of baicalin-induced apoptosis in CRC cells (Figure 5). Baicalin could induce CRC cell apoptosis through the mitochondrial pathway mediated by ROS generation, survivin and Bcl-2 inhibition as well as caspase-3, caspase-8, caspase-9 induction, the Wnt pathway activated by Dickkopf1 and lipoprotein receptor-related protein-6 and the Notch pathway activated by Notch1, hairy and enhancer of split 1 and Jagged generation.

Promoting cellular senescence of CRC cells . Cellular senescence, as a fundamental cellular destiny, is a cellular state where cells are unable to proliferate but still survive, which is different from the G0 stationary phase and terminal differentiation state. It is a state of highly stable cell- cycle arrest that occurs in diploid cells which limits the lifespan of these cells. By inhibiting growth, cellular senescence limits the replication of senescent or damaged cells and thus can act as a tumor suppressor [46]. Treatment with baicalin significantly induces senescence in colon cancer cells. In addition, baicalin upregulates the expression level of progesterone-induced decidual protein in HCT-116 cells accompanied by activation of the Ras/Raf/MEK/ERK and p16INK4A/Rb signalling pathways. These phenomena also occur in response to the antioxidant effect of baicalin. In addition, ectopic expression of progesterone in HCT-116 cells significantly induces the activity of senescence-associated galactosidase in tumor cells regulated by the Ras/Raf/β/ERK signalling pathway [46]. Interference or suppression of Depp by RNA can effectively counteract baicalin-induced growth inhibition, senescence, and cell cycle arrest in cancer cells. Importantly, baicalin treatment significantly inhibited tumor growth by inducing tumor cell senescence via upregulation of Depp and activation of the Ras/Raf/MEK/ERK signalling pathway in a xenograft mouse model of human colon cancer [47]. In addition to baicalin treatment, it has also been found that the hypoxia-responsive protein Depp has a positive regulatory effect involving the regulation of the Ras/Raf/MEK/ERK signalling pathway and the inhibitory effect of other antioxidants, such as curcumin and sulforaphane, on human colon carcinoma, leading to tumor cell senescence.

The above findings suggested that baicalin can lead to senescence through upregulation of Depp and activation of its downstream Ras/Raf/MEK/ERK and p16INK4A/Rb pathways [47]. However, the exact molecular mechanism of baicalin-mediated upregulation of Depp expression is still unknown. The occurrence of epithelial-mesenchymal transition (EMT) during treatment confers greater invasiveness to tumor cells and promotes adverse progression of tumors. Senescent cells release senescence-associated secretory phenotype factors such as IL-6 and IL-8 into the tumor microenvironment, which induce the onset of epithelial-mesenchymal transition and thus play a role in promoting metastasis and invasion [48]. In summary, the induction of senescence in tumor cells is important for the treatment of colorectal cancer, and it also has the adverse effects of promoting tumor metastasis and drug resistance, so cellular senescence is a “double-edged sword”, which has both advantages and disadvantages for the treatment of colorectal cancer.

Effect of baicalin on the invasion and metastasis of CRC cells. In the occurrence, progression and metastasis of cancer, epithelial-mesenchymal transition can confer invasiveness and migration abilities on epithelial tumor cells, which in turn promotes their dissociation from surrounding tissues, degradation of the basilar membrane, invasion of blood vessels, and eventually metastasis to distant tissues [49]. When cancer cells undergo genetic alterations, the expression of oncogenes and cancer suppressor genes associated with tumorigenesis and progression changes. Morphologically, epithelial cells shift to fibroblastic or mesenchymal cells and then acquire the ability to migrate, leading to infiltrative growth of the tumor and metastatic abilities [50]. The above studies suggest that the epithelial-mesenchymal transition process is an important mechanism by which tumor cells acquire a malignant phenotype. In addition, EMT not only improves the motility and invasive ability of tumor cells but also improves the anti-apoptotic ability. Once cancer cells undergo epithelial-mesenchymal transition, they initiate an autocrine state promoting their own growth, and at the same time, lose targets that are removed by the organism, thus escaping apoptosis. Accordingly, EMT may play a critical role in the formation of highly invasive tumor cells.

Moreover, it has been reported that the process of EMT promotes the formation of stem-like cells, that is, highly invasive cells formed by epithelial-mesenchymal transition have stem cell-like properties, and this ability to self-renew can promote cancer cell dissemination and the formation of secondary tumor foci [50]. TGF-β1, a transforming growth factor, can promote cell migration and invasion by initiating the EMT process. Baicalin not only reversed the EMT process but also inhibited the EMT process induced by TGF-β1/Smad in RKO, HCT-116 and SW480 cells [51]. However, the molecular mechanism of how baicalin acts on the TGF-β1/Smad signalling pathway still needs to be investigated.

Some studies have suggested that EMT induces the production of cells with stem cell properties and, to some extent, the clone formation

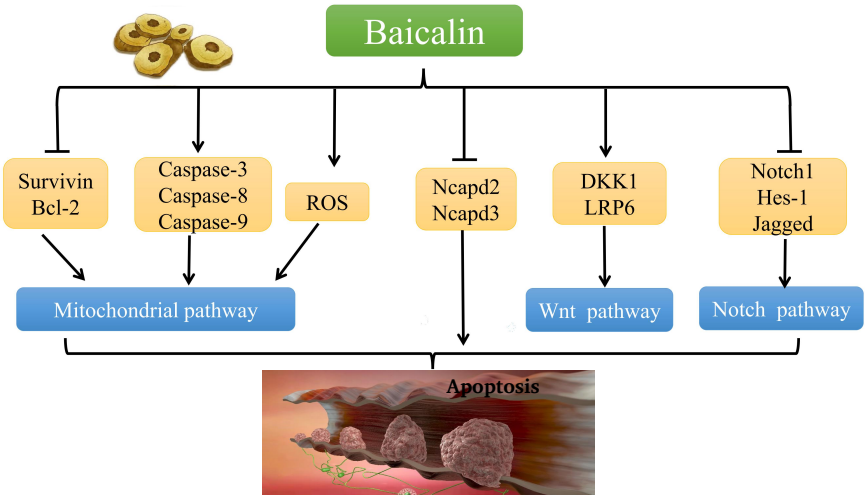

Figure 5 Schematic illustration of the molecular mechanism of baicalin-induced apoptosis in CRC. Baicalin induced apoptosis in CRC cells through multiple mechanisms, such as increasing the ROS, caspase-3, caspase-8 and caspase-9 level, inhibiting survivin and bcl-2 expression, upregulating Dickkopf1, lipoprotein receptor-related protein-6 that activating Wnt, as well as inhibiting the level of Notch1, Hairy and enhancer of split 1, Jagged, Ncapd2 and Ncapd3. CRC, colorectal cancer; ROS, reactive oxygen species.

ability can reflect such properties [52, 53]. Baicalin intervention significantly reduced the protein levels of stem cell markers, including CD133, CD44, Sox2, Oct4, and Nanog, both in adherent cultured cells and growth of suspended tumors and spheres, and baicalin intervention reduced the differentiation ability of those spheres [54]. Since tumor stem cells are associated with drug resistance in cancer, namely, drug tolerance, it will be useful to further investigate whether baicalin in combination with traditional chemotherapeutic drugs can better treat cancer. Although the results of in vitro experiments showed that baicalin could inhibit stem cells of colorectal cancer, the impact of baicalin on tumor stem cells in vivo still needs to be explored.

The AKT signalling pathway is another important dysregulated pathway in the occurrence and progression of cancer, and the elevated protein and phosphorylation levels in the AKT pathway are critical for tumor growth and metastasis in colon cancer [55]. In our study, we added the PI3K inhibitor LY294002 to the culture medium in the presence of baicalin and found that the latter did not significantly inhibit cell growth, proliferation, migration, or invasion, while cell formation ability was further suppressed [56]. This suggests that baicalin may exert its anti-colon cancer effect by inhibiting the PI3K/AKT/GSK-3β pathway. Further results also showed that baicalin can inhibit PI3K, AKT, and GSK-3β mRNA levels, but the addition of LY294002 had little effect on I3K, AKT, and GSK-3β mRNA levels in cells [56]. In summary, baicalein has a role in regulating the biological behaviour of colon cancer cells, including inhibition of proliferation, migration invasion, angiogenesis, and promotion of apoptosis, and this effect of baicalin may be related to its ability to inhibit the PI3K/AKT/GSK-3β pathway. However, further in vivo experiments are needed to verify the anti-colon cancer effects of baicalin, and the mechanism underlying its regulation of AKT-related pathways still needs to be further investigated.

Intestinal flora. The relationship between dysbiosis of intestinal flora and the progression of colon and rectal cancers has attracted widespread attention [57]. We investigated the effects of the human intestinal microbiota on the biotransformation of baicalin by observing its complete metabolism. After eight hours of incubation, baicalin was fully metabolized to baicalein. Other data also suggest that the human intestinal microbiota can effectively metabolize baicalin to baicalein [58]. Since water immersion may structurally modify baicalin, we tested whether water only (without microbiota) could convert baicalin to baicalein. It was shown that water immersion without enzymes could not induce conversion after eight hours of incubation. The inhibition of colorectal cancer cell proliferation in vitro was verified using an in vivo transplantation model in nude mice. Baicalin showed limited inhibitory effects on the proliferation of some cancer cell lines. However, baicalein showed significant inhibition effects on proliferation in all tested cancer cell lines, especially on HCT-116 human colon cancer cells. The results of in vivo antitumor experiments support our in vitro experimental data. It has been found that baicalein has significant S-phase blocking and proapoptotic effects on HCT-116 cells. Baicalein can induce the activation of caspase-3 and caspase-9 [58]. Computerized molecular docking showed that baicalein combined with Ser251 and Asp253 residues at the active site of caspase-3 formed hydrogen bonds, while it interacted with Leu227 and Asp228 residues in caspase-9 through their hydroxide radicals.

Malignant tumor are complex, intractable and systemic diseases caused by multiple genes, links, and factors. Modern medicine principles indicate that any biological phenomenon has a biomolecular basis. Combined with the principles of systems biology, the process of emergence and progression of cancer can be understood as a series of interactions of related molecular events and changes in distribution and composition. As the “second genome” of the human body, the intestinal flora must undergo a series of molecular events closely related to tumor prevention, occurrence and progression continuously every day. These molecular events involve many proteins, cytokines and signalling pathways in the intestinal flora microenvironment. Future research should integrate modern technologies such as metagenomics, macro-transcriptomics, next-generation high-throughput sequencing technology, gene chips, and germ-free mice to explore how baicalin exerts its effects on the prevention and treatment of colon cancer through intestinal flora and to identify key strains and specific flora. The results from these studies will provide a theoretical basis for the adjuvant treatment of intestinal flora dysbiosis in colorectal cancer with Chinese medicine, as well as new ideas and methods for the clinical treatment of colorectal cancer.

Anti-inflammatory activity. It is estimated that approximately 20% of human cancers are caused by chronic inflammation. Recent studies have shown that inflammatory media play a direct role in the malignant progression of colon cancer [59]. Baicalin can significantly reduce the levels of inflammatory mediators, including myelin peroxidase activity, tumor necrotizing factor α, IL-1β, and Th1-related cytokine IL-12 levels. In addition, the beneficial effects of baicalin appear to be related to regulating the balance of Th17 and Treg cells [60]. We found that baicalin could significantly reduce the number of Th17 cells and the levels of Th17-related cytokines (IL-17, IL-6) and retinoic acid receptor-related orphan receptors. In contrast, the number of Treg cells, Treg-related cytokine conversion growth factors-β, IL-10, and forkhead box P3 were increased [60]. Our results suggest that the anti-colon cancer effect of baicalin may be associated with regulating the balance of TNBS-induced ulcerative colitis Th17/Treg cells against inflammation. Another study has demonstrated that baicalin, by inhibiting PGE2 production and COX-2 activity, can effectively suppress tumor cell growth [61]. The findings provide a molecular basis by which baicalin can be used to treat inflammatory disorders as well as cancers. However, the exact connection at the molecular level between COX-2 and inflammatory disorders and carcinogenesis remains unclear.

Synergy between baicalin and other antitumor drugs. Baicalein and baicalin are active components of *Scutellaria baicalensis* Georgi with a broad range of antitumor activities. However, it is not clear how and whether baicalein and baicalin inhibit colon cancer. Dou et al. have shown that baicalein and baicalin significantly inhibit the growth, migration and proliferation of human colon cancer cells. Furthermore, both compounds can induce cell cycle blockade and inhibit cancer cell formation and migration. One of the potential mechanisms is that baicalein and baicalin can induce colon cancer cell apoptosis and senescence respectively. We have further shown that baicalin induces tumor cell senescence because it inhibits the expression of telomerase reverse transcriptases in tumor cells and that MAPK ERK and p38 signalling pathways are involved in the regulation of apoptosis and senescence of colon cancer cells mediated by baicalein and baicalin [62]. Recent studies have also shown that human Treg cells selectively regulate specific MAPK p38 and ERK1/2 signalling pathways in the responding T cells, thereby controlling the senescence of the responding T-cells [63, 64]. Our current research shows that the MAPK, ERK, and p38 signalling pathways are involved in apoptosis and senescence induced by baicalein and baicalin. Studies from other research groups have shown that many other signalling pathways are also involved in the regulation of apoptosis of cancer cells mediated by baicalein and baicalin, including PI3K/AKT, mTOR, and NF-κB signalling pathways [62]. However, these studies have not taken into account the mutation states of KRAS/NRAS/BRAF in colon cancer cells, which are important for clinical outcomes and survival in cancer patients [65, 66]. Therefore, continuous efforts should include the identification of molecular regulatory mechanisms and disease-inducing links between these signalling pathways, which involve antitumor effects mediated by baicalein and baicalin. Baicalein and baicalin inhibit the conversion of growth factors-β-1-mediated epithelium-mesenchyme transformation of human mammary epithelial cells. We used human colon cancer cells for in vivo research in a humanized mouse model of xenografts, further proving that baicalein and baicalin can induce tumor apoptosis and senescence to inhibit the growth of colon cancer [14]. These data

show that baicalein and baicalin have a strong anticancer effect on human colon cancer and may become a new effective target drugs for this disease.

Clinical application. The Huangqin decoction (Decoction of *Scutellaria baicalensis* Georgi), first recorded in *Treatise on Cold Damage Diseases* (Zhong-Jing Zhang, about 150–219 C.E.), is composed of *Scutellaria barbara*, Licorice, Paeonia and *Ziziphi Jujubae*. In recent years, the innovative anticancer drug PHY906 based on *Scutellaria baicalensis* decoction has attracted much attention, and preclinical and clinical studies have shown that it is effective for the treatment of colorectal cancer, and can relieve the toxic side effects of some chemotherapy drugs [67, 68]. PHY906 has been studied in clinical trials to treat colon cancer in the United States and China. The clinical application of the baicalin monomer in cancer therapy has not been reported.

New dosage form of baicalin. Baicalin's clinical efficacy is seriously affected by its poor water solubility and low degree of oral biological utilization. However, researchers have used new technologies to produce novel preparations, such as baicalin nanocrystals [69]. They are designed to improve baicalin's bioutilization in pharmacotherapy. However, we have found in the literature review that there are still shortcomings in the new preparations; for example, a single drug-carrying system was characterized by a low envelope rate and instability. With the aid of compound drug-carrying systems, that is, combining two or more kinds of drug-carrying systems to give full advantages of each system, the physical and chemical properties of baicalin can be further improved for better clinical efficacy. Taking into account the pharmacological properties of baicalin and the properties of the lesion site, we can choose the appropriate type of preparation to assist clinical treatment. For instance, because of the high permeability and long retention effect of the colorectal tumor site, a more targeted baicalin agent can be prepared.

Conclusion

With the increasing interest in the antitumor mechanisms of Chinese medicine, more research has been conducted on the efficacy and mechanism of the active ingredients of baicalin. As described in Table 1, a large number of studies have shown that baicalin has a strong effect on a variety of colorectal cancer cells through various target molecules and mechanisms. The pharmacological effect involves blocking the cancer cell cycle, inducing tumor cell apoptosis, and inhibiting tumor metastasis. There has been an increasing trend of in vivo research in recent years. However, the following research aspects require further examination. First, more in-depth research is needed on the molecular mechanisms of baicalin in cancer chemotherapy. The anticancer activity of baicalein is generally higher than that of baicalin, and the antitumor mechanisms of the two are similar. Since the two can be transformed into each other in vivo, their pharmacodynamics can be studied on the tumor model of the body to clarify the active ingredients and effective concentrations that play a specific role in the body. Research on antitumor preparations of baicalin and bacilein, used alone or with other medicines in treating colorectal cancer should be intensified. Moreover, studies involving baicalin intervention in CRC via multiple targets and pathways have shown that it has good application prospects. However, there are problems such as low selectivity and insufficient in-depth research on the anti-colorectal cancer mechanism. Therefore, whether the improvement and functional modification groups of *Scutellaria baicalensis* screen out derivatives with higher selectivity and lower cytotoxicity necessitates further exploration. Finally, clinical trials regarding baicalin and the specific molecular mechanisms involved should be investigated more extensively. In summary, baicalin is promising as an anti-colorectal cancer therapeutic drug and should be developed for clinical trials.

Table 1 Molecular targets of Baicalin in colorectal cancer

	Experimental model	Biological response	Invovled genes/pathway	Reference
Animal model	HCT-116-xenograft	↑ G(2)/M stage arrest	↑ DNA damage	[12, 13]
Cell lines	HCT-116, HT29 and SW480 cells	G(1)/S stage arrest	↑ DNA damage	[14, 17, 16]
Cell lines	SW480 cells	↑ Apoptosis	↑ Caspase 3 and Caspase 9, ↓ Bcl-2	[18, 19]
Cell lines	SW620 cells	↑ Apoptosis ↑ Reactive oxygen species	↑ Caspase 3, Caspase 8 and Caspase 9	[21]
Animal model	BALB/c nude mice	↓ Tumor volume	repair gene hMLH1	[15]
Cell lines	HCT-116 cells	↑ Apoptosis	↑ Caspase 3, ↓ Survivin	[23]
Cell lines	HT-29 cells	↑ Apoptosis	↓NCAPD2 and NCAPD3	[26]
Cell lines	DLD1 and HCT-116 cells	↑ Apoptosis	↓Wnt	[29]
Cell lines	SW480 cells	↑ Apoptosis ↓ Proliferation	↓ Notch	[37, 38]
Cell lines	SW480 cells	↑ Apoptosis ↓ Cell growth	↓ specificity protein 1	[42]
Cell lines	HT-29 cells	↑ Apoptosis	↓ c-Myc and oncomiRs	[43]
Cell lines	HCT-116 cells	↓ Cellular senescence	↑ Ras/Raf/MEK/ERK	[44, 45]
Cell lines	RKO, HCT-116 and SW480 cells	↓ Invasion	↓ TGF-β1/Smad	[50]
Cell lines	SW620 cells	↓ Invasion ↓ Proliferation	↓ PI3K/AKT/GSK-3β	[54]
Cell lines	KM-12 and HCT-15 cells	↓ Cell growth	↓ prostaglandin E2, ↓ cyclooxygenase-2	[61]

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