BBA - Molecular Basis of Disease 1868 (2022) 166281

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Review

Therapeutic potential of melatonin in colorectal cancer: Focus on lipid metabolism and gut microbiota

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ARTICLE INFO

Keywords:
Melatonin
Colorectal cancer
Lipid metabolism
Gut microbiota

ABSTRACT

Colorectal cancer (CRC) is one of the most common gastrointestinal malignancies. The occurrence and development of CRC are complicated processes. Obesity and dysbacteriosis have been increasingly regarded as the main risk factors for CRC. Understanding the etiology of CRC from multiple perspectives is conducive to screening for some potential drugs or new treatment strategies to limit the serious side effects of conventional treatment and prolong the survival of CRC patients. Melatonin, a natural indoleamine, is mainly produced by the pineal gland, but it is also abundant in other tissues, including the gastrointestinal tract, retina, testes, lymphocytes, and Harder's glands. Melatonin could participate in lipid metabolism by regulating adipogenesis and lipolysis. Additionally, many studies have focused on the potential beneficial effects of melatonin in CRC, such as promotion of apoptosis; inhibition of cell proliferation, migration, and invasion; antioxidant activity; and immune regulation. Meaningfully, gut microbiota is the main determinant of all aspects of health and disease (including obesity and tumorigenesis). The gut microbiota is of great significance for understanding the relationship between obesity and increased risk of CRC. Although the current understanding of how the melatonin-mediated gut microbiota coordinates a variety of physiological and pathological activities is fairly comprehensive, there are still many unknown topics to be explored in the face of a complex nutritional status and a changeable microbiota. This review summarizes the potential links among melatonin, lipid metabolism, gut microbiota, and CRC to promote the development of melatonin as a preventive and therapeutic agent for CRC.

1. Introduction

Colorectal cancer (CRC) is a common malignant tumor; it is the third most common cancer morbidity, and its mortality rate is the second highest among all cancers. Nearly 0.9 million CRC patients die each year, accounting for approximately 10% of all confirmed cancer cases and cancer-related deaths worldwide [1,2]. CRC has significant sex differences, with morbidity and mortality rates among men approximately 25% higher than those among women. In addition, there are significant regional differences in CRC: the incidence in developed countries is almost 3 times that in developing countries [3–5]. The global occurrence of CRC is expected to increase by 60%, reaching more than 2.2 million new cases and 1.1 million deaths by 2030 [6]. Liver metastasis has been shown as one of the leading causes of death in patients with CRC. From an anatomical point of view, there is an extra set of blood circulation pathways between the large intestine and liver than

Abbreviations: CRC, colorectal cancer; GIT, gastrointestinal tract; HFD, high-fat diet; EC, Enterochromaffin; IBD, inflammatory bowel disease; TG, triglyceride; CPT1A, Carnitine palmitoyl transferase-1A; FAO, fatty acid oxidation; RORα, retinoic acid-related orphan α; MSCs, Mesenchymal stem cells; PPARγ, peroxisome proliferator-activated receptor gamma; HSL, hormone-sensitive lipase; Nrf2, nuclear factor erythroid 2-related factor 2; MMPs, matrix metalloproteinases; EMT, epithelial-mesenchymal transition; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; VEGF, vascular endothelial growth factor; HIF-1, hypoxia-inducible factor-1; STAT3, signal transducer and activator of transcription 3; ET-1, endothelin-1; ROS, reactive oxygen species; ER, endoplasmic reticulum; SOD, superoxide dismutase; GSH, glutathione; mφ, macrophages; SCFA, short chain fatty acids; ACL, ATP-citrate lyase; ACC, acetyl-CoA carboxylases; FASN, fatty acid synthase; SCD1, stearoyl-CoA desaturase-1; ATGL, adipose triglyceride lipase; MAGL, monoacylglycerol lipase; UCP1, uncoupling protein 1; CPT1, carnitine palmitoyl transferase 1; FFA, free fatty acids; SFA, saturated fatty acid.

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https://doi.org/10.1016/j.bbadis.2021.166281
Received 3 March 2021; Received in revised form 24 August 2021; Accepted 26 September 2021
Available online 2 October 2021

other general organs, which is the portal vein system. One side of the portal vein is in the gastrointestinal tract (GIT), and the other side is in the liver. Its task is to transport nutrients absorbed from the GIT to the liver. Liver cells with high levels of de novo fatty acid synthesis and lipid accumulation in lipid droplets may have an adaptive advantage because they store the necessary fuel to allow the metastasis and colonization of cancer cells. The reduction of free fatty acid (FFA) synthesis and FFA transport to mitochondria also indicates a tendency to limit metastasis by lowering intracellular reactive oxygen species (ROS) levels. This reduces DNA damage, which would lead to mutations that allow CRC cells to colonize the liver that are different from their origin [7]. Based on current studies, targeting de novo lipogenesis would be a potential strategy to prevent and delay the growth of CRC liver metastases [8,9]. The liver environment might lead CRC cells to undergo metabolic reprogramming, which enhances lipid metabolism and promotes growth of CRC liver metastases. Patterns and trends in CRC morbidity and mortality are associated with current levels of human development, including genetic makeup, population aging, dietary behavior, and environmental factors [10,11]. Here, we mainly focus on the roles of lipid metabolism and the gut microbiota in CRC. An increasing number of epidemiological studies and animal experiments have confirmed that lipid metabolism disorders are one of the risk factors for CRC. In particular, high-fat diet (HFD)-induced dysbacteriosis has been increasingly regarded as one of the main risk factors of obesity-related CRC, suggesting that the interaction between gut microbiota and obesity plays an important role in the development of CRC [12]. To understand the etiology of CRC from multiple angles, it is necessary to explain how obesity regulates the composition of the gut microbiota, metabolic capacity, and colonic cavity performance and to further screen for some potential drugs. It may be beneficial to reduce the adverse side effects of chemotherapy for CRC, including cytotoxicity, drug resistance, and tumor recurrence and metastasis.

Melatonin (*N*-acetyl-5-methoxytryptamine) was discovered by A. Lerner in 1958 [13], which is mainly synthesized in the pineal gland [14]. It is an indole substance first isolated from an extract of pig pineal gland that could discolor frog skin. Tryptophan is a key precursor in melatonin synthesis. It plays an important role in cell growth, maintenance and coordination of changes in the external environment. It is a synthetic precursor for a large number of microorganisms and host metabolites [15]. Tryptophan in the body has two sources: one is the endogenous amino acid that is broken down by tissue protein, and the other is the exogenous amino acid that is digested and absorbed from the diet. There are three main pathways for the metabolism of tryptophan in the body: one is directly converted into several molecules through the gut microbiota, including ligands for the aryl hydrocarbon receptor (AhR), and the other is the kynurenine pathway. The third is the production pathway of serotonin (5-HT) in enterochromaffin cells through the action of tryptophan hydroxylase 1 (TpH 1) [16]. Melatonin is also a metabolic product of tryptophan. After obtaining tryptophan from blood, pineal cells form 5-HT catalyzed by tryptophan hydroxylase (TPH) and aromatic amino acid decarboxylase (AAAD), and then melatonin is produced by the action of *N*-acetyltransferase (AA-NAT) and acetylserotonin-*O*-methyltransferase (ASMT) [17]. Tryptophan metabolism is one of the important mechanisms for cancer to evade immune surveillance [18]. 95% of dietary tryptophan is metabolized through the kynurenine pathway [19]. Indoleamine 2,3 dioxygenase 1 (IDO1), as a critical rate limiting step, has key roles in limiting adaptive immune responses in CRC. Several studies have indicated that decreased tryptophan levels and increased kynurenine pathway metabolites, suggesting increased IDO1 activity in CRC patients [20,21]. High IDO1 expression in tumor draining lymph nodes is related to a decrease in the 5-year survival rate of CRC patients [22]. In addition, compared with normal human colonic epithelial cells, CRC cells DLD1, HT29, HCT116, HCT15, RKO, LoVo were more sensitive to the consumption of tryptophan. Blocking enzymes in the kynurenine pathway leads to priority death of established CRC cells and transformed colon organoids [20]. Collectively, tryptophan is reported to be down-regulated in current studies of CRC, which involves suppressing the generation of immunosuppressive T cells that regulate CRC development.

The well-known function of melatonin is mainly to regulate the circadian rhythm to improve the quality of sleep. The most important characteristic of melatonin is that it is the strongest endogenous free radical scavenger found so far, delaying aging. The basic function of melatonin is to participate in the antioxidant system and prevent oxidative damage to cells. A large number of clinical and experimental studies have shown that as an endogenous neuroendocrine hormone, melatonin has a direct and indirect physiological regulatory effect on the central nervous system, has a therapeutic effect on sleep disorders, depression and mental diseases, and has a protective effect on nerve cells. Melatonin can regulate cellular immunity and humoral immunity, as well as the activity of a variety of cytokines. In addition, melatonin also has a regulatory effect on the human cardiovascular system, respiratory system, digestive system, and urinary system. It is also involved in immunity, weight loss, and anti-inflammatory and anti-cancer functions [23–25].

Multiple studies have shown the synthesis of melatonin in other organs/tissues than the pineal gland, including immune system, gastrointestinal, retina, skin, and cochlea. Here, we will focus on extrapineal melatonin produced in the retina, immune system and GIT. The synthesis pathway in these places is similar to that of the pineal gland, but differentially regulated. Interestingly, melatonin locally produced in the retina would be essential for the functioning of the eye. Rods, cones, retinal ganglion cells (RGCs), and retinal pigment epithelium (RPE) seem to constitute the main source of melatonin in the eye in a circadian manner [26–28]. The retinal circadian clock has been widely reviewed, which seems to ensure adaptive functioning, and ultimately regulates the alternation between light and dark adaptation effects [29,30]. Furthermore, melatonin produced by the retina also has a key function as an antioxidant, such as counteracting ischemic damage in RPE cells [31]. Additionally, melatonin has been previously documented to be locally synthesized by different immune cells and tissues, such as in bone marrow, spleen, thymus, and mast. Although direct data on the immunomodulatory function of pineal melatonin are abundant, there is much evidence that local melatonin may play an on-site protective role, including protecting these vulnerable hematopoietic cells from oxidative stress, enhancing the immune capacity of lymphocytes [32], and activating and differentiating T cells [33]. These immune cells and tissues could rhythmically produce their own melatonin, and may supplement the one from the pineal gland through autocrine or paracrine mechanisms, so the possibility of circulating uptake would not be discarded.

Increasing numbers of studies have focused on the positive regulation of melatonin in the gastrointestinal system. GIT is the most important source of melatonin outside of the pineal gland, and its level is 400 times higher than that of the pineal gland [34]. However, only a small proportion of melatonin in the GIT comes from the pineal gland [35], and it is mainly synthesized by intestinal enterochromaffin (EC) cells [36] that secrete serotonin through the gastrointestinal mucosa. In 1976, it was confirmed that these cells contain the enzyme hydroxyindole-*O*-methyltransferase, which is necessary for the synthesis of melatonin from tryptophan. Interestingly, the level of melatonin in the gut is not dependent on the pineal gland. The concentration of melatonin in the intestines of pinealectomy rats was found to be unaffected [37]. It has also been speculated that the light and physiological independence of gastrointestinal melatonin is highly concentrated in colorectum [38], not obviously released into the blood, but that it can act as a reservoir in the pineal gland secreting indoleamine to supply melatonin [39]. It is noteworthy that unlike pineal melatonin, gastrointestinal melatonin levels increase dramatically after feeding [40], suggesting that gastrointestinal melatonin synthesis and secretion are regulated by ingestion and food composition [41]. Furthermore, the concentration of melatonin in the intestine is age related, i.e., it decreases with aging [39].

With the development of technology, various methods for the determination of melatonin have been developed. Various well-known researchers have localized the melatonin following immunohistological techniques, and measured the concentration of melatonin by radioimmunoassay as well as gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC) and ELISA. To be able to answer what type of melatonin (pineal or extrapineal) exerts antitumor effects in CRC, different advanced experimental methods would be needed to distinguish the activity of both origins. Some studies have described a coordinated interaction between the pineal gland and GIT-derived melatonin in intestinal function. In general, pineal melatonin, as a neuroendocrine hormone, is released into the serum cyclically, following the circadian rhythm. It mainly affects the nervous system, immune system, and endocrine system through humoral and neurosecretory pathways to exert a biological effect, that is, it is necessary to regulate gastrointestinal function. From a local perspective, gastrointestinal tissue can synthesize and secrete a certain level of melatonin, but it is not excessively released into the blood. It mainly affects the activities of surrounding cells and self-secreting cells through paracrine and autocrine pathways [25]. The synthesis and rapid absorption of this local melatonin enhance intestinal immune activity, prevents harmful compounds or toxins from being produced by the gut microbiota, and inhibits colon diseases and dysplasia. In addition, considering the concentration of indoleamine required to act on different processes, we can infer what type of melatonin, depending on its source, acts in different ways. Therefore, those pathways that require a higher concentration of melatonin than the blood (secreted by the pineal gland), and those that target the photophase but not the scotophase will probably be determined by local melatonin production. CRC is a good example of how the pineal and extra-pineal melatonin work together to form a properly organized organ physiology. Taken together, in any case, as previously discussed, it is likely that pineal melatonin regulates local melatonin production. The pineal and extrapineal melatonin secretion dance is not random and should be finely regulated. However, great efforts remain to be required to understand the separate roles and possible interplay of pineal and extrapineal melatonin during light and dark phases. Additionally, as an important regulator of gastrointestinal motility and inflammation, melatonin plays a positive role in the control of many intestinal diseases, especially intestinal inflammation and cancer. Melatonin could enhance intestinal immune function, regulate peristalsis and change gut microbiota to inhibit the occurrence and development of IBD or CRC and other related diseases, which is mainly manifested in inducing apoptosis and reducing cell proliferation, migration and invasion. Similarly, melatonin, as an adjunct to chemotherapy, ameliorates sleep and physical function of CRC patients, resulting in improving their quality and life expectancy. Therefore, it is not surprising that melatonin has the potential to suppress gastrointestinal cancers.

Many host processes depend on the gut microbiota, such as glycolipid metabolism, the regulation of intestinal motility, and immune protection. Our groundbreaking work has shown that melatonin reduces obesity by affecting the gut microbiome [42]. Subsequent studies also confirmed the above arguments [43,44]. It is particularly important to establish a link between gut microbiome dysfunction and several diseases, including obesity, inflammatory bowel disease (IBD), and CRC. Further work is essential to determine the mechanisms by which melatonin regulates physiological function and its effect on related metabolic disorders through the gut microbiota. Here, first, we outline the interrelationship between melatonin and the occurrence of CRC. Then, we discuss the potential mechanisms of lipid metabolism and gut microbiota in the occurrence and development of CRC. Finally, we elucidate the possible relationships among melatonin, the intestinal microbiome, lipid metabolism, and CRC, in order to lay the foundation for melatonin as a promising drug for cancer prevention and treatment.

2. Melatonin and colorectal carcinogenesis

Melatonin has an anti-tumor effect and has synergistic and detoxifying effects in adjuvant tumor therapy. Tamarkin et al. first observed that melatonin could significantly inhibit the development of mammary tumors in Sprague-Dawley rats, which pioneered the anti-tumor research on melatonin [45]. Recent studies have shown that melatonin may be a potential candidate for tumor prevention and treatment, including in breast cancer [46], melanoma [47], prostate cancer [48], oral cancer [49] and thyroid cancer [50]. Mechanistically, melatonin favors regulating sex hormone pathways, modulating growth factors, influencing cell cycle or cell metabolism, interfering with calmodulin and tubulin functions, increasing intercellular gap junctions, antioxidant and immune-enhancing effects. A comprehensive understanding of the mechanism of melatonin would contribute to its further clinical application.

Increasing numbers of studies have focused on the positive role of melatonin in regulating gastrointestinal tumors, including CRC. In an interesting experiment, melatonin was still detected in the lower gut after pinealectomy, and its level tended to be stable, suggesting that melatonin could be synthesized locally [51]. Subsequent studies have also explained that melatonin secretion is widespread in the GIT, which has a wide range of melatonin binding sites. It is suggested that it is related to the complicated regulation of gastrointestinal physiology. The distribution of melatonin is related to the density of intestinal EC cells [36] and has regional differences, with the highest concentration in the rectum and colon, and the lowest in the jejunum and ileum [52]. After the addition of exogenous melatonin, the accumulation of melatonin was most significant in the colon and rectum [53]. In addition, although melatonin has a significant daily rhythm in the pineal gland, plasma, pancreas, kidney, spleen, and duodenum, the daily pattern of melatonin levels in the colon is irregular, without a characteristic night-time increase in hormone concentration [54]. Especially during the day, higher levels of melatonin in the colon than in serum increase the immune activity of the intestine and stimulate free radical scavenging, thereby reducing nitro-oxidative stress and improving the metabolic rate and immune defense [55]. These findings suggest that melatonin may have a potential inhibitory effect on CRC progression.

Melatonin participates in many physiological processes, including regulating sleep/wake cycle, immune regulation, regulating cell apoptosis, anti-tumor and anti-oxidation. Many of the physiological and pharmacological effects of melatonin are mediated by melatonin receptors [56]. Three types of melatonin receptors have been confirmed: membrane receptors (including MT1 and MT2), cytoplasmic receptors (MT3) and nuclear receptors (RZR/RORα). Melatonin receptors mediate many cellular effects, including changes in intracellular cyclic nucleotides (cAMP and cGMP), changes in calcium levels, activation of certain protein kinase C subtypes, and regulation of potassium channels, calcium channels, and intracellular steroid receptor localization. In the digestive system, melatonin would accumulate in the stomach and colon after systemic administration, and contains a large number of melatonin receptors in the GIT [57]. It has been confirmed that oncostatic effect of melatonin involvement of acting via both MT2 and RZR/ROR nuclear receptors on murine Colon 38 cancer [58,59]. Additionally, MT1 and MT2 are involved in mediating the anti-tumor cell proliferation mechanism. Several analytical studies have reported a statistically significant downregulation in the levels of MT1 and MT2 mRNA and the protein expressed in tumor mucosa of CRC patients [60,61]. Interesting, the human CRC lines, including Caco-2, HT-29, and DLD-1 also showed lower MT1/MT2 expression in comparison with normal human colon FHC line [60]. Moreover, melatonin could inhibit HT-29 cell viability by enhancing cytotoxicity and apoptosis by the activation of MT3 receptor [62]. However, the relationship between CRC and the status quo of melatonin receptors is unclear. It remains open and needs more conclusive evidence.

Moreover, epidemiological studies have suggested that the circadian

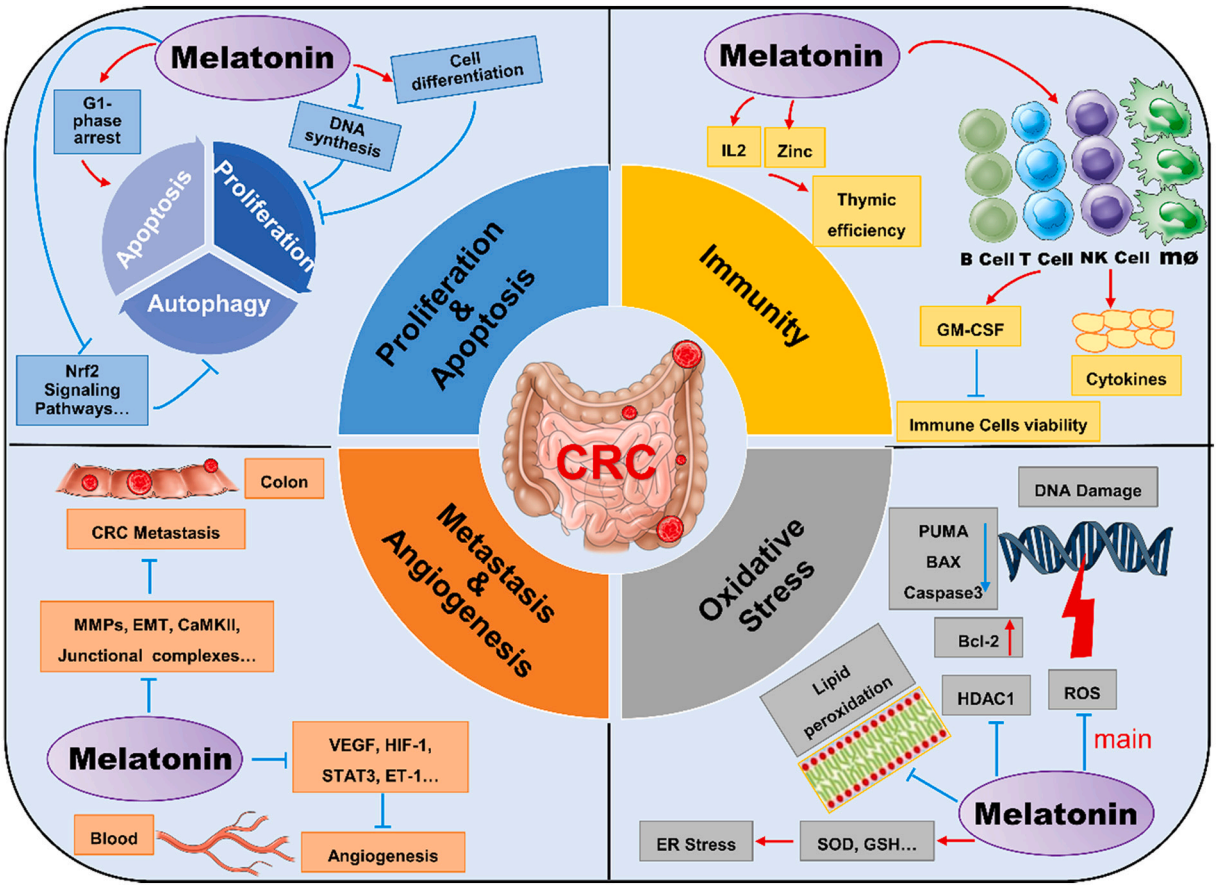

Fig. 1. Schematic representation of potential mechanisms linking melatonin to inhibition of the progression of CRC. Melatonin would inhibit the development and progression of CRC through a variety of ways, including increasing apoptosis and reducing proliferation, migration and invasion, as well as antioxidation and immune regulation. The red arrow represents promotion, and the blue arrow represents inhibition.

rhythm of melatonin in the blood of CRC patients is disordered, and a disruption of melatonin homeostasis would increase the risk of CRC in humans [63–66]. It has been reported that the plasma melatonin levels of the control group and CRC patients were 0.23 ± 0.04 nmol/L and 0.1 ± 0.02 nmol/L during the day, and 0.39 ± 0.11 nmol/L and 0.16 ± 0.05 nmol/L at night, respectively [67]. There is a significant decrease in the peak amplitude of melatonin secretion and overall output in CRC patients [68]. Although descriptions of melatonin levels varied between different groups [38,69], melatonin destruction was closely associated with CRC in general, suggesting that melatonin may be explored as potential therapeutic agents on CRC. Lissoni et al. first carried out clinical trials to investigate the effects of melatonin on cancer in 1987 [70]. This study selected 19 patients with advanced solid tumors, including CRC. And preliminary study would indicate that melatonin treatment (20 mg/day, intramuscularly) may have a positive effect on improving their performance status and quality of life. Interestingly, in clinical studies in 1990 (14 patients) [71] and 2003 (30 patients) [72], it was found that melatonin would have an adjunctive effect in metastatic CRC patients resistant to fluorouracil. A 1993 study [73] found that the clinical research of 35 patients, including CRC, suggested the possible synergistic anti-cancer effect of melatonin and IL-2, which was also confirmed by a subsequent study by Brivio et al. in 1995 [74]. More recently, large clinical studies investigated the effects of melatonin (20 mg/day) in 1440 patients with untreatable advanced solid tumors (279 patients with CRC) in 2002 [75] and 370 cancer patients in 2007 [76]. These clinical evidences demonstrate that the melatonin may play a positive role in the supportive care.

Collectively, melatonin has been shown to play an essential regulatory role in various animal models and clinical studies of colon inflammation or cancer. We summarized the anti-cancer effects of melatonin in the following aspects. To begin with, melatonin regulates cell proliferation, apoptosis, and autophagy, and the homeostatic balance of these three activities is important for maintaining the integrity of the intestinal mucosa [77]. Melatonin mainly inhibits the proliferation of HT-29 cells by inhibiting DNA synthesis and promoting cell differentiation [78]. In addition, melatonin alters the cell cycle program by increasing G1 phase arrest, thereby activating apoptosis [79]. An interesting aspect of the anti-tumor effect of melatonin is its ability to induce apoptosis. This response is only observed in cancer cells, which leads to an effective reduction in tumor volume, thereby improving the patient's clinical condition [20,80–82]. It is worth noting that melatonin activates the ERK1/2 signal of normal cells. On the contrary, it could inhibit ERK1/2 in cancer cells, hinder the proliferation of cancer cells, and potentially break the resistance of cancer cells to cytotoxic therapy [83]. Melatonin has an anti-apoptotic effect in normal cells, but has a pro-apoptotic effect in many cancer cells. These dichotomous behaviors have aroused the interest of researchers. Therefore, fully clarifying the signaling pathways and molecular targets of melatonin-induced apoptosis of CRC cells lays the foundation for choosing efficient therapeutic targets. Moreover, melatonin treatment attenuated the tumorigenesis of CRC in mice by regulating autophagy and the Nrf2 signaling pathway [84]. However, the mechanism by which melatonin mediates the dual role of autophagy in tumorigenesis remains to be further explored.

Melatonin could also inhibit cell migration and anti-angiogenesis. Melatonin inhibits the migration of RKO cells through the p38/MAPK signaling pathway [85,86]. It is worth noting that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), which can regulate the

Table 1
Summary of in vitro studies investigating the effects and mechanisms of melatonin on CRC.

Effects	Authors	Year	Cell types	Treatment	Mechanism
Proliferation	Farriol M, et al. [111]	2000	CT-26 cells	1, 2 and 3 mM	A moderate, but significant antiproliferative action of melatonin on this non-hormone-dependent cell line
	Winczyk K, et al. [59]	2002	Colon 38 cells	10^{-7} M	Acting MT(2) and RZR/ROR nuclear receptors
	García-Navarro A, et al. [78]	2007	HT-29 cells	10^{-3} M	Reducing the production of nitric oxide
	Santoro R, et al. [112]	2013	HCT116 cells	1 μM	Inducing melatonin receptor's ability
	León J, et al. [93]	2014	Caco-2 and T84 cells	1 mM	Inhibiting endothelin-1 expression and secretion through the inactivation of FoxO-1 and NF-κβ
	Ji G, et al. [113]	2021	HCT116, LoVo, SW480, SW620, HT-29 and DLD-1 CRC cells	0.1, 0.5, 1, 1.5 or 2 mM	Upregulating apoptosis-related proteins cleaved caspase-3 and cleaved PARP
Apoptosis	Batista AP, et al. [114]	2014	Caco-2 cells	1.56 and 0.78 μg/mL	Inducing the generation of reactive oxygen species (ROS)
	Hong Y, et al. [79]	2014	HCT-116 cells	10 μM	Inducing G1-phase arrest at the advanced phase
	Wei JY, et al. [115]	2015	LoVo cells	1 mM	Inducing HDAC4 nuclear import mediated by CaMKII inactivation
	Chovancova B, et al. [80]	2017	DLD1 cells	0.1, 1, and 10 μM	Regulating the type 1 sodium/calcium exchanger, and type 1 IP3 receptor
	Yun CW, et al. [116]	2018	SNU-C5/WT cells	1 mM	Regulating PrPC-dependent pathway
	Lee SJ, et al. [117]	2018	HCT116 cells	1 μM	Induced by *Vibrio vulnificus* VvhA via melatonin receptor 2 coupling with NCF-1
	Ji G, et al. [113]	2021	HCT116 and LoVo CRC cells	2 mM	Upregulating apoptosis-related proteins cleaved caspase-3 and cleaved PARP
Autophagy	Hong Y, et al. [79]	2014	HCT-116 cells	10 μM	Inducing G1-phase arrest at the advanced phase
Migration	Zou DB, et al. [86]	2015	RKO cells	25 μM	Down-regulating myosin light chain kinase expression through cross-talk with p38 MAPK
	Liu Z, et al. [85]	2017	RKO cells	2.5 mM	Down-regulating Rho-associated protein kinase expression via the p38/MAPK signaling pathway
Anti-angiogesis	Park SY, et al. [118]	2010	HCT 116 cells	1 mM	Inhibiting HIF-1α stabilization under hypoxia in HCT116 cells
	León J, et al. [93]	2014	Caco-2 and T84 cells	1 mM	The inactivation of FoxO-1 and NF-κβ
Antioxidant	Bułdak RJ, et al. [119]	2015	HCT 116 cells	10^{-6} M	Decreasing antioxidant capacity
DNA damage	Liu R, et al. [120]	2013	HCT-15 cells	1 nM	Affecting genes involved in DNA damage responsive pathways
	Santoro R, et al. [112]	2013	HCT 116 cells	1 μM	Regulating melatonin receptor's ability
	Mannino G, et al. [121]	2019	Caco-2 cells	50 mM	Reducing IL-1β-induced DNA-breakage

proliferation, migration, and invasion of HCT116 cells through the ERK1/2 and p38 pathways, may be a potential key mediator for the development and metastasis of human colon tumors [87]. Interestingly, melatonin could inhibit CaMKII activation through multiple pathways (including P21, RB, MAPK, ERK1/2, and AKT1) [88–90] to produce positive effects [91]. In addition, the anticancer effect of melatonin in the inhibition of CRC angiogenesis has been studied. Angiogenesis promotes the activity of aggressive tumors, including tumor growth, metastasis, and invasion [92], and the factors involved are vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1). Notably, ET-1 is a survival factor in CRC and is closely related to the proliferation, apoptosis, and angiogenesis of cancer cells. Melatonin is negatively correlated with the expression of ET-1 [93] and is responsible for inhibiting the related carcinogenic effects mediated by this factor, thereby blocking the development of CRC.

In addition, melatonin is a powerful nonenzymatic antioxidant that protects normal cells from oxidative damage to DNA and cell membranes caused by carcinogens, thereby inhibiting the development of cancer [94]. Melatonin is not only a direct detoxifier for substances such as nitric oxide, peroxyl radicals, peroxynitrite, and hydroxyl radicals, but also reduces severe DNA damage caused by unstable oxygen radicals and nitrogen radicals, thus reducing tumor initiation and incidence. It also regulates oxidoreductases such as catalase, lipoxygenase, paraoxidase, and superoxide dismutase [95]. This antioxidant effect is particularly important in protecting cells from environmental factors, such as radiation-induced chromosome damage and mutations [96].

Finally, melatonin may play a role in the neuroendocrine-immune-tumor network, thereby affecting tumor growth. Melatonin could inhibit the development of CRC by regulating immunity [97]. The immune function of melatonin includes humoral immunity and cellular immunity [33,98], which promote the production of antibodies and increase the amount and activity of cytokines. Specifically, melatonin stimulates the expression of IL-2 and IL-12 in macrophages and induces lymphocytes to secrete the cytokines IL-2 and IL-6 through nuclear receptors; in addition, melatonin and its oxidation products can inhibit the expression of TGF-α and IL-8 in neutrophils mediated by lipopolysaccharide; and melatonin may increase the number of lymphocytes and enhance the cytotoxic effect of NK cells [99].

In subsequent studies, it would strengthen the understanding of melatonin on the epigenetics of CRC. The epigenetic type of CRC is highly sensitive to environmental impacts, which affects genome stability through DNA synthesis, repair, and methylation. Melatonin may serve as an additional environmental input to CRC cells that may influence the expression of target epigenetic genes, such as the ability to regulate genomic instability due to the activation of retrotransposons, thereby reducing the risk of carcinogenic mutations [100].

Collectively, it is of practical significance to fully reveal the relationship between melatonin and CRC. The potential mechanisms of how melatonin inhibits the progression of CRC are shown in Fig. 1. Interestingly, melatonin would diminish the metabolic reprogramming of cancer cells [101,102]. It is well known that the mitochondria of healthy cells generate melatonin. Melatonin in mitochondria would inhibit pyruvate dehydrogenase kinase (PDK) activity and break metabolic reprogramming that leads to aerobic glycolysis. The putative deprivation of melatonin in cancer cells, especially during the day, is related to the interruption of melatonin biosynthesis in the mitochondria. The elevated level of circulating melatonin in the pineal gland at night might inhibit PDK activity, and drive a shift from aerobic glycolysis to mitochondrial oxidative phosphorylation (OXPHOS) [103]. The evidence that has been reported suggests that cancer cells are different from

Table 2
Summary of in vivo studies of melatonin effects on CRC.

Year	Subjects	Dose	Route	Timing	Observation
2000 [123]	Rats	20 mg/L in water, 5 days/ week	PO	6 months	Decrease in the area of lymphoid infiltrates in the colon mucosa of tumor-bearing rats
2000 [126]	Rats	20 mg/L in water, 5 days/ week	PO	6 months	The numbers of CD8+ lymphocytes and Fas-positive cells increased sharply
2001 [130]	Mice	10 and 100 μg/animal	SC	6 days	RZR/ROR receptors in the pro-apoptotic effect of melatonin
2002 [122]	Rats	1 μg/animal, 5 days/ week	SC	6 months	Inhibitory effect of peptide Epitalon on colon carcinogenesis
2002 [58]	Mice	25 μg/animal	SC	10 days	Nuclear RZR/RORalpha receptors participate in the oncostatic action of melatonin
2002 [59]	Mice	25 μg/animal	SC	6 days	Oncostatic effect of MLT depends on acting via both MT(2) and RZR/ROR nuclear receptors
2003 [127]	Rats	1 μg/animal, 5 days/ week	SC	6 months	Inhibitory effect of Epitalon on carcinogenesis
2011 [125]	Rats	10 mg/kg	IP	14 days	A great potential to control the preneoplastic patterns induced by constant light
2016 [84]	Mice	1 mg/kg	PO	8 and 18 weeks	Modulating autophagy and Nrf2 signaling pathways
2017 [124]	Mice	5 mg/kg	PO	10 d	Decreasing “oncogenic” and increasing “onco-suppressive” ROS
2018 [129]	Mice	10 mg/kg	IP	Once daily at days 0, 1 and 2	Increased radiosensitivity
2018 [106]	Mice	20 mg/kg	IP	Pre-treatment at 24 and 72 h prior to exposure	Antioxidant effects
2003 [72]	Human	20 mg	Orally	9 weeks	Enhancing the therapeutic activity
2019 [128]	Human	20 mg	Orally	5 days a week for 28 days	Prevent or minimize the unfavorable effects of radiotherapy on blood cell count reductions

healthy cells in many aspects, including metabolism, gene regulation, and stress response, which causes these cells to respond differently to the same concentration of melatonin compared with healthy cells [104,105]. In addition, by stimulating and inhibiting the antioxidant system, melatonin may sensitize tumor cells while protecting normal tissues [106]. The antioxidative effect of melatonin may well protect normal cells and the entire organism from the systemic effects of chemotherapy [7]. However, since most of the in vitro on melatonin has been only conducted on cancer cells, and not on normal cells concurrently, there is no reason to believe that, in general, this protective effect would not be conferred equally on tumor cells. With the increasing attention and research on melatonin in the field of oncology, we need to understand the anti-tumor effect of melatonin in depth. This information would be valuable for the potential application of melatonin in the treatment of neoplastic diseases.

More significantly, melatonin, as an endogenous molecule, is well tolerated at physiological concentrations. It was originally used as a sleep aid, and no untoward effects were reported in this regard. There is a great number of scientific evidence that melatonin has proven anticancer and therapeutic effects at physiological ($\leq 10^{-9}$ M) and supraphysiological (10^{-9}–10^{-2} M) concentrations. Although melatonin might exacerbate some symptoms in a few cases, most studies have shown very low toxicity over a wide range of concentrations [107–110]. Looking back, we could find that the verification of melatonin effects in the experimental system is often performed at concentrations (in the millimolar range) higher than those found inside cells and tissues, so some direct effects reported in the clinical literature may be the product of melatonin concentrations much higher than those in cells [100,109]. One of the mechanisms involved may be the actions of its receptors to make melatonin play a protective role. To better determine the safe pharmacological ranges and risk assessment of melatonin, further conclusive data to understand optimal dosing protocols and new formulations are necessary. Moreover, more randomized clinical studies are needed to corroborate the oncoprotective capacity of melatonin. This information would help us to better understand the differences in the sensitivity of cancer cells to melatonin and to further elucidate the synergistic effects of melatonin and other drugs, such as radioprotection and radiosensitization, to achieve the key goals of selective use of melatonin and individualized treatment of tumors.

The following summarizes in vitro (Table 1) [59,78–80,85,86,93,111–121] or in vivo (Table 2) [58,59,72,84,106,122–130] studies investigating the effects and mechanisms of melatonin on CRC.

3. Lipid metabolism and the gut microbiota in CRC

3.1. Lipid metabolism in CRC

Lipids are the general term for fats (triglyceride, TG), fatty acids, cholesterol, and phospholipids. Lipids are not only the energy source and structural components of various cell membranes but also play important roles in cell signal transduction, energy metabolism, material transport, and cell proliferation [131,132]. For TG, fatty acids, one of their decomposition products, are mainly involved in the regulation of CRC. It has been found that the total plasma concentrations of saturated, monounsaturated, and polyunsaturated derivatives in CRC patients were significantly lower than those in healthy subjects [133]. Excessive intake of saturated fatty acid (SFA) could lead to obesity and induce insulin resistance, thereby increasing the risk of CRC. It is worth mentioning that polyunsaturated fatty acids mainly include ω3 PUFA and ω6 PUFA, which are antagonistic to each other. ω6 PUFA could be biosynthesized into eicosanoids (such as PGF2), which can regulate tumor cell proliferation, apoptosis, and invasion by activating tumor epithelial cell receptors, as well as inducing epithelial cells to secrete tumor growth factors and inflammatory mediators to promote tumor angiogenesis, and ultimately promote the tumorigenesis of CRC. However, ω3 PUFA plays an opposite role by competitively inhibiting ω6 PUFA [134–136]. In addition, regarding cholesterol, studies have shown that HDL and apo-A concentrations are negatively correlated with CRC risk [137], while VLDL levels are positively correlated with it [138]. Changes in cholesterol levels could affect fat structure, regulate immune function and cell signaling, and ultimately lead to tumorigenesis [139,140]. In addition, cholesterol is eventually converted into bile acid, which can also promote the development of CRC [141]. What is more, as an important component of biofilms, phospholipid is involved in cell metabolism and signal transduction, which in turn affects cell proliferation and apoptosis, and ultimately affects the development and progression of CRC. Studies have shown that cyclic phosphatidic acid

(CPA) could not only inhibit the invasion and metastasis of cancer cells by regulating mitosis but also inhibit the activity of cyclic nucleotide phosphodiesterase 3B (PEB3B) and stimulate an increase of intracellular cAMP levels to activate the PKA pathway and ultimately inhibit the carcinogenesis of CRC [142,143]. Thus, various types of lipids are correlated with CRC, but the specific mechanisms need further exploration.

Lipid metabolism (including lipid synthesis, lipid utilization, and lipolysis) is associated with the development and progression of various cancers [144]. Many studies have shown that the increased de novo synthesis of fatty acids in tumor cells is a significant feature of cancer development and is inversely related to the prognosis of various types of tumors. The main purpose of the increase in lipid production is to synthesize more cell membrane lipids to support the rapid proliferation of cancer cells and the soaring energy demand, suggesting that the activation of FA synthesis is necessary for carcinogenesis and tumor cell survival. The key regulators of lipogenesis, SREBP, acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase 1 (SCD1), and other genes are significantly upregulated in tumors, with lymph node metastasis in patients with CRC, TNM stage and poor prognosis being closely related. The mechanisms involved are complex and varied. It has been reported that SREBP1 may promote the invasion and metastasis of CRC by stimulating the phosphorylation of NF-κB p65 and by increasing the expression of MMP7 [145]. FASN may enhance the proliferation and metastasis of SW480 and HCT116 cells by regulating the AMPK/mTOR pathway [146]. SCD1 accelerates the progression of CRC by promoting epithelial-mesenchymal transition (EMT) [147]. In addition to de novo synthesis, lipid uptake from the external environment is another key way that cells obtain fatty acids to participate in tumor progression. The key gene involved, CD36, could transport fatty acids into cells and play a key role in the growth, metastasis, and EMT of cancer [148].

Furthermore, fatty acid β-oxidation plays a key role in tumor growth. Carnitine palmitoyl transferase-1A (CPT1A), the enzyme responsible for mitochondrial uptake of FAs, could mediate the activation of fatty acid oxidation (FAO) and provide abundant energy to increase the transfer capacity of HCT15 and HCT116 cells, and CPT1A might therefore be regarded as a potential target for the treatment of metastatic CRC [149]. Notably, when cells have too much lipids, they are converted to TG and cholesterol esters in the endoplasmic reticulum, where they are stored as lipid droplets (LDs) [150]. Chemotherapy-induced LD content in CRC cell lines (SW620, LoVo, HCT116, HCT8, SW480 and HT29) was positively correlated with the expression of lysophosphatidylcholine acyltransferase 2 (LPCAT2), suggesting that LD accumulation has a potential effect on CRC chemoresistance [151,152]. Thereafter, lipolysis could also be utilized by cancer cells to varying degrees to meet their needs for fatty acids. It has been reported that a lipolytic activator, lacking α/β hydrolase domain-containing 5 (Abhd5), also known as comparative gene identification 58 (CGI-58), is negatively correlated with CRC. Silencing Abhd5 could induce EMT by inhibiting the AMPKα-p53 pathway, implying that cancer cells regulate the development of CRC by inhibiting Abhd5-mediated intracellular lipolysis [153]. These findings have explained the important relationship between lipid metabolism and the development of CRC, but the specific mechanism needs to be supplemented in subsequent studies.

Increasing amounts of evidence have shown that abnormal lipid metabolism is closely related to the occurrence and malignant transformation of CRC. The first point focuses on the effect of HFD-induced lipid metabolism disorders on CRC. It was observed that HFD could increase the content of colorectal bile acids, damage the intestinal barrier, change the gut microbiota, further affect the proliferation and apoptosis of colonic epithelial cells, and ultimately promote the tumorigenesis of CRC [154]. HFD could also affect the density and function of intestinal endocrine cells (such as Paneth cells and EC cells). Excessive fat intake could reduce the number of Paneth cells and decrease intestinal resistance to bacteria, thus promoting the occurrence of intestinal tumors [155,156]. Similarly, an HFD alters innate immunity and adipose tissue secretion to cause chronic low-grade inflammation, and dysbiosis could cause increased numbers of *Escherichia coli* (*E. coli*), decreased mucus layer thickness, increased intestinal permeability, upregulated Nod2 and Tlr5 expression, and TNF-α secretion. These changes enhance the colonization ability of adherent invasive *E. coli* in the colorectum, thereby further aggravating inflammation and promoting the tumorigenesis of CRC [157].

Abnormal lipid metabolism is regarded as one of the key causes of CRC. Gut microbiota in patients with abnormal lipid metabolism may be involved in the progression of CRC. It has been reported that in CRC patients with dyslipidosis, the abundance of *Escherichia*, *Shigella*, and *Streptococcus* increased, while the abundance of *Clostridium XIVa* and *Ruminococcaceae* decreased. Furthermore, *Selenomonas*, *Clostridium*, *Bacteroidetes*, *Slackia*, *Burkholderiales* and *Veillonellaceae* were closely related to CRC patients complicated with dyslipidosis [158]. One explanation may be that dysregulation of the gut microbiota, including changes in the abundance of *Bacillus bifidus*, *Lactobacillus* and *Enterococcus*, could affect the activity of cholesterol oxidase and liver fat synthetase, as well as the redistribution of triglycerides and cholesterol, leading to lipid metabolism disorders [159–161]. On the other hand, abnormal lipid metabolism induced by HFD would reduce the absorption of intestinal energy and nutrients, and affect the intestinal microenvironment and change the abundance and composition of microbial populations [162]. CRC can be influenced by the metabolic output of the entire microbiota. In addition, some specific bacteria and microbial metabolites may contribute to the development of CRC [158,163]. Further clinical and experimental studies would provide stronger evidence that CRC, lipid metabolism and gut microbiota are interrelated and interact with each other. The subsequent focus has been on the effect of lipid oxidative stress and its products on CRC. Disturbance of lipid metabolism could enhance the lipid oxidative stress response. Low-density lipoprotein could promote the synthesis of intracellular ROS in SW480, LoVo or RKO cells, as well as the oxidation of proteins, lipids, and DNA, leading to redox imbalances and oxidative stress, and the secretion of inflammatory molecules or anti-inflammatory molecules, which in turn promotes the progression and deterioration of CRC [164]. Furthermore, another important point suggests that tumor-associated adipocytokines could interfere with the progression of CRC. A clinical investigation showed that the serum leptin levels of overweight Chinese patients with CRC were significantly higher than those of colectomy patients [165]. It has been reported that leptin can interact with the CRC related promoter MPS-1 to activate the JNK/c-Jun signaling pathway, thereby inducing the growth of tumor cells and inhibiting the apoptosis of RKO and HCT116 cells [166]. In our previous study, we demonstrated that mature adipocyte conditioned medium promotes the proliferation and migration of SW480 and C26 cells through inhibition of the nuclear receptor retinoic acid-related orphan α (RORα) [167]. Another important adipocyte factor, adiponectin, can mediate its anti-tumor and anti-angiogenic effects by binding to its receptors Adipo-R1 and Adipo-R2, and inhibit the development and progression of CRC [168,169].

In summary, a relationship between lipid metabolism and CRC has been increasingly confirmed, so the question is, will lipid-lowering drugs prevent or limit the occurrence of CRC while reducing lipids? At present, there are some studies on statins and the risk of CRC. In addition to solely lowering cholesterol to achieve cancer suppression, there may be some other mechanisms involved. Further research on related drugs is needed to provide new insight for the prevention and treatment of CRC.

3.2. Gut microbiota in CRC

The gut microbiota is an important component of the intestinal microecosystem, consisting of more than 1000 species of bacteria. It is well known that the gut microbiota is involved in the processes of digestion and absorption, immune regulation, and material metabolism in the human body. It plays an important role in protecting the intestinal

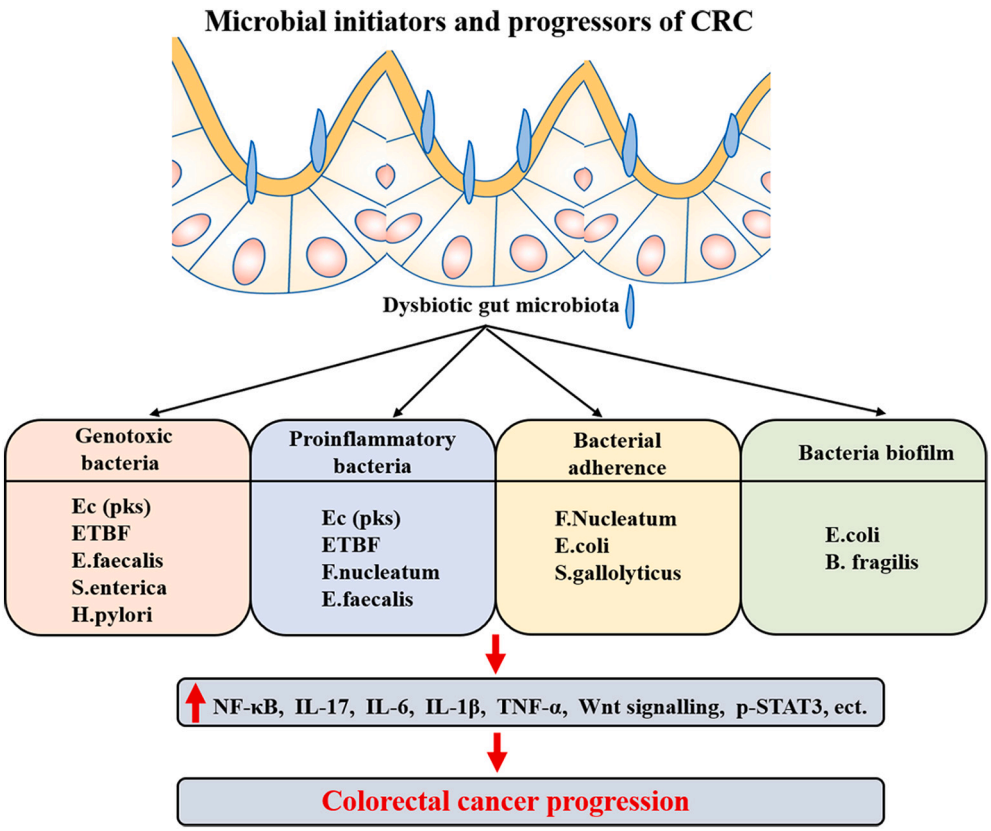

Fig. 2. Summary of the role of the microbiota in CRC initiation and progression. Microbiota in the gut show different kinds of expression patterns. Physiological and physical factors can influence the bacterial growth. It describes a variety of ways in which bacteria may trigger or promote CRC tumorigenesis, including direct genotoxicity of specific bacteria, and pro-inflammatory effects induced by specific microbes, overall microbiome dysregulation, and/or colonic biofilms.

mucosa, maintaining the normal functions of the intestinal tract and even the body. Due to the differences in the transit time, pH value, oxygen content, nutrients, and secretory functions of the intestinal tract in different segments, the colonization of bacteria in different segments of the intestine is different [170]. The spatial distribution characteristics of intestinal microorganisms are as follows: from the proximal to the distal end of the intestine, the number and diversity of microorganisms increase in sequence, and the bacterial abundance and diversity in the colon are the greatest. It is worth mentioning that the bacterial levels in the colon are higher than those in the small intestine [171] and that the colon has approximately 12 times more cases of cancer than the small intestine [172], indicating the potential role of the gut microbiota in the tumorigenesis of CRC.

A change in the gut microbiota is related to the occurrence of colorectal adenoma. As early as 1975, the Weisburger group first reported an association between the gut microbiota and CRC in sterile rats [173]. At present, a growing number of clinical and animal model studies have linked the two, proving that changes in the structure and quantity of gut microbiota occur throughout the development of CRC. The abundances of *Erysipelotrichia* and *Fusobacteria* are increased in the feces from patients with serrated polyps. The abundance of *Bilophilia*, *Desulfovibrio*, *Corynebacterium*, and *Phascolarctobacterium* in the stools of patients with conventional adenoma is increased significantly [174,175]. In the intestinal mucosa of patients with adenocarcinoma, the number of bacteria of *Proteobacteria* such as *Pseudomonas*, *Helicobacter*, and *Acinetobacter*, is increased [176]. Some pathogenic bacteria and their toxins, such as *Fusobacterium nucleatum*, *E. coli*, and *Bacteroides fragilis*, show an increasing trend with the evolution of CRC (polyp - adenoma - adenocarcinoma) in the human gut [177–179]. In summary, the composition of the microbiome during the development of CRC may vary according to the stage of the disease.

These data suggest an unexpected link between CRC and tumorigenic bacteria, such as *Shigella*, *Fusobacterium nucleatum*, and *Bacteroides fragilis* [161,180–182]. These pathogens cause millions of cases of foodborne diseases in the United States every year, and the cost of hospitalization and lost productivity is enormous. In developing countries, diseases caused by these pathogens are not only more prevalent, but also associated with higher mortality rates. To begin with, dysregulation of the gut microbiota causes abnormalities in signal transduction pathways to induce intestinal mucosal inflammation and other immune responses, making the mucosa prone to inflammatory adenoma or cancer formation. It has been shown that the gut microbiota activates macrophages on the intestinal mucosa to trigger the innate immune response, which is manifested in the overexpression of pattern recognition receptors such as TLRs on the surface of macrophage membranes. These receptors, upon receiving stimulatory signals from the gut microbiota, could induce immune cells to secrete large amounts of inflammatory cytokines such as IL-1, IL-6, IL-8, and TNF-α, thereby promoting the development of CRC [183]. Similarly, *Fusobacterium nucleatum* could inhibit the activity of $CD4^+$ T cells in CRC, making tumor growth more uncontrolled [184].

It has been concluded that the progression of CRC is determined by the tumor microenvironment, which includes a variety of immune cells. The mechanism involved may be related to the cytokines produced by immune cells and related signaling pathways, such as the Wnt, Notch, and TGF-β pathways, which could affect the self-renewal of colonic mucosal epithelial cells. Interestingly, the transcription activators NF-κB and STAT3 affect colon repair and immune repair. Specifically, NF-κB is a key regulator of innate immunity and inflammatory responses and is closely related to the initiation and development of intestinal tumor-like lesions [185]. When IKK-β was inhibited in the NF-κB pathway of intestinal epithelial cells, the incidence of CRC significantly decreased

[186]. STAT3 increases the susceptibility of the colonic epithelium to colitis, and it could also increase the incidence of CRC by mediating the secretion of IL-6 and IL-1 [187].

In addition, some gut microbes could damage the DNA of intestinal epithelial cells through their direct action or the production of toxic metabolites, causing gene mutations or chromosomal instability, which leads to abnormal cell growth and the formation of tumors. For example, the bacteriocin produced by *Enterobacteriaceae* is a hybrid nonribosomal peptide-polyheteroside compound encoded by the 54 kDa polyoxin synthase (Cpks) genotoxic island that can also cause DNA double-strand breaks and chromosome instability [161,188,189]. In a recent paper in Science, Wilson et al. [190] proved that colicin produced by bacteria alkylates DNA in the body, and this work provides new insights into its role in the pathogenesis of CRC [190].

Furthermore, the bile acid-bacteria interaction leads to the occurrence and development of CRC. Abnormal levels of bile acids can cause intestinal microflora disorders, and dysregulation of gut microbiota could also affect a series of processes such as bile acid synthesis and metabolism. Bile acids as substrates can produce carcinogenic metabolites under the action of intestinal bacterial enzymes, such as secondary bile acids, hydrogen sulfide, and ROS, thereby promoting the development and progression of CRC [191,192].

The gut microbiota has also been gradually applied to the prevention and treatment of CRC. First, regulating the gut microbiota is one important means of preventing and treating CRC. Using probiotic preparations to maintain the stability of the intestine can reduce the occurrence of intestinal inflammatory diseases. The next step is to adjust the gut microbiota by improving the diet structure. We could regulate the structure of the gut microbiota through food and medicine, inhibiting the growth of pathogenic bacteria, affecting the host's metabolism, and achieve the purpose of preventive treatment. The subsequent step is to rebuild the normal intestinal microecosystem by transplanting a functional microbiota from healthy human feces.

Most importantly, screening followed by early diagnosis and treatment are the main ways to prevent CRC, which might be achievable by detecting the gut microbiota. Through the examination of fecal bacteria in patients, IBD could be diagnosed and intervened early to achieve the prevention and treatment of CRC. In recent years, a large number of results have been obtained in the study of the gut microbiota and the metabolites of CRC, but before we can use these findings in clinical practice, there are still some limitations that need to be overcome, such as the sample size of the studies, the technology of the studies, the statistical methods used and the diagnostic limitations.

In summary, based on the developments concerning the gut microbiota in CRC, along with the current research hotspots of intestinal microecology and metabolomics, regulating the gut microbiota is expected to be a means of preventing or treating CRC. The metabolism of exogenous compounds by the gut microbiota may affect the efficacy of CRC treatment. Understanding the composition and metabolism of the gut microbiota is conducive to the selection of treatment regimens and monitoring strategies to improve therapeutic efficacy. The role of the microbiota in CRC initiation and progression is shown in Fig. 2. It also suggests the possibility of the use of bacteria expressing specific genes or producing specific metabolites for the treatment or prevention of CRC.

4. Melatonin and lipid metabolism

Melatonin, which has emerged as a central regulator of lipid metabolism, is associated with adipogenesis, lipogenesis, and lipolysis.

Recent studies have observed that melatonin is responsible for adipogenesis. Mesenchymal stem cells (MSCs) are induced to differentiate into preadipocytes and further develop into mature adipocytes through some signaling factors, that is, adipogenesis. It is a complex process regulated by cell morphology, gene expression, or hormone sensitivity, which mediates the contradictory effects of melatonin on adipogenesis. Some groups think melatonin suppresses preadipocyte differentiation and adipogenesis [44,193–198]. Melatonin-treated (10^{-3} M) 3T3-L1 preadipocytes could reduce differentiation through inhibiting the transcriptional activity of the CCAAT/enhancer-binding protein β (C/EBPβ) promoter [193], which could well-regulate the expression of the main controllers peroxisome proliferator-activated receptor gamma (PPARγ) and the CCAAT/enhancer binding protein alpha (C/EBPα) involved in the complete adipogenesis program [199]. Further evidence suggested that melatonin (50 nM) inhibited the phosphorylation of C/EBPβ by blocking the ERK/GSK-3β sites, to ultimately decrease adipogenesis in human MSCs [194]. Another interesting observation was that the downregulation of PPARγ could mediate the effect of melatonin (10^{-4} M) on suppressing adipogenesis, while increasing osteogenic differentiation by promoting Runx2 expression in human MSCs (hMSCs), which was the opposite of adipogenesis [195]. Furthermore, Knani et al. recently further reported the potential mechanism of melatonin in regulating the balance of osteogenic/adipogenic differentiation of hMSCs, including affecting apoptosis and DNA damage [196]. Likewise, some studies have evaluated the roles of melatonin in the adipogenic potential of human adipose-derived stem cells (hADSCs) [197,198] and found melatonin significantly inhibited the expression of marker genes involved in the regulation of adipogenesis, including PPARγ and aP2, as well reduced intracellular fatty acid synthesis and lipid accumulation. Interestingly, our group recently reported that melatonin decreased lipogenesis gene expression to suppress lipid accumulation in L-02 cells and lipid infiltration in the mouse liver [44]. These findings support the hypothesis that melatonin exerts anti-adipogenic effects to regulate lipid homeostasis.

In contrast, other groups have proposed that melatonin is a positive regulator of preadipocyte differentiation and adipogenesis [200–202]. Previous studies reported that melatonin induced 3T3-L1 preadipocytes proliferation by activating the MT2 receptor, which may be involved in the improvement of antioxidant enzyme activity and the weakening of lipid peroxidation [203]. Similarly, melatonin (1 mM) significantly promoted PPARγ, C/EBPβ, and C/EBPα expression, as well as increased TG accumulation by the MT2 receptor in bovine intramuscular preadipocytes (BIPs) [202]. Interestingly, other reports have also demonstrated a similar positive effect in 3T3-L1 cells [200,201]. Consistent with these findings, melatonin administration significantly increased the expression of adipogenesis genes, upregulated PPARγ and C/EBPα expression, and increased adipogenic differentiation with large lipid droplets and TG accumulation.

Combined with the above findings, the differential effect of melatonin-mediated adipogenesis has not been well resolved. In these previous studies, first of all, there are differences between different groups for inducing mature adipocytes. It is well known that adipogenesis is a highly coordinated process that is time-dependent. For example, it takes 8 days for 3T3-L1 preadipocytes to become mature adipocytes [200,201], while hADSCs [197] and hMSCs [194] require a longer differentiation time (21 d or 28 d). Considering that the formulation of the inducer used (such as insulin, dexamethasone, or isobutylmethylxanthine) differs, variations in the cell lines and the cell differentiation methods used could affect the final adipogenesis results. Furthermore, the treatment time and dosage of melatonin were different among studies. For example, the concentration applied to hMSC cells was 50 nM to 0.1 mM [194,195], while the incubation concentration for 3T3-L1 cells was 1 nM to 0.1 M [193,200,201]. These distinct treatments might regulate different effectors in vivo or in vitro to modulate key transcription factors such as PPARγ, C/EBPα, and C/EBPβ, thereby modulating adipogenesis. Taken together, the mechanism of melatonin-mediated adipogenesis remains unclear, and more research is needed to explain how melatonin regulates adipogenesis and differentiation of adipocytes in the future.

In addition to adipogenesis, melatonin also plays an important role in lipogenesis, which is an anabolic pathway for TG accumulation that regulates the fundamental metabolic process in adipocytes. Studies have shown that melatonin administration can decrease a series of hepatic

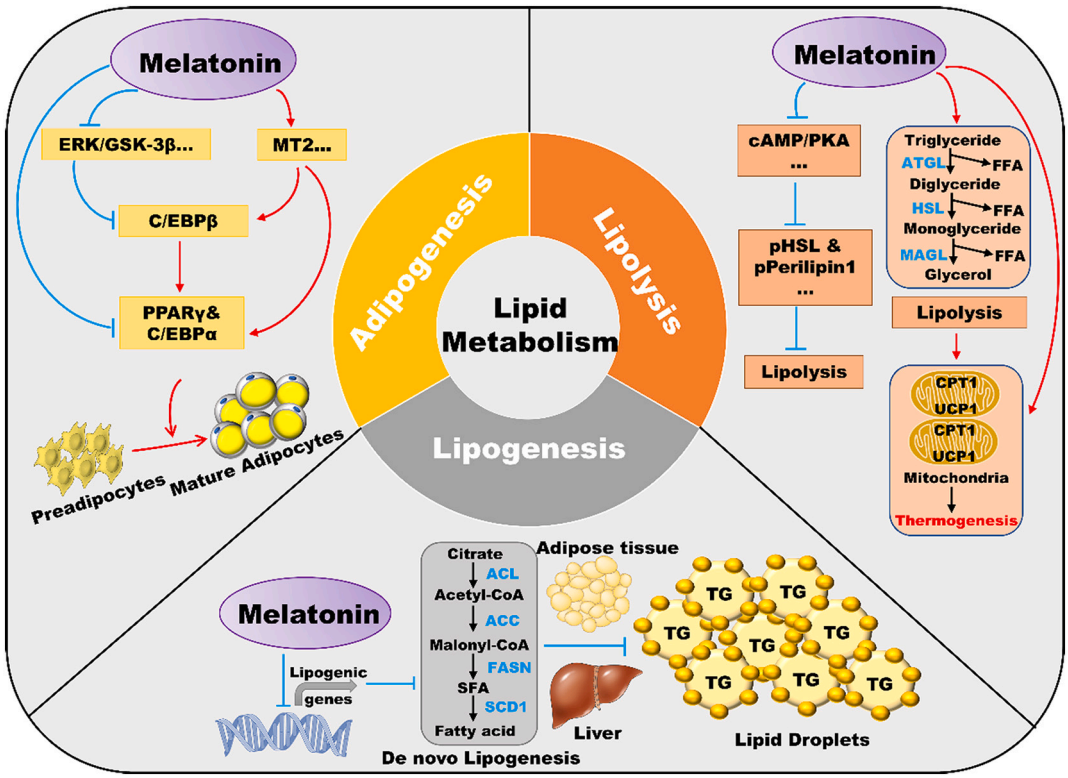

Fig. 3. Schematic representation of possible mechanisms of melatonin in lipid metabolism. Melatonin can promote or inhibit the expression of C/EBPβ and play different roles in adipogenesis. In addition, melatonin can inhibit de novo lipogenesis to decrease the formation of lipid droplets. Furthermore, it is generally believed that melatonin can promote lipolysis and increase thermogenesis. However, paradoxically, melatonin can also inhibit lipolysis through the cAMP/PKA signaling pathway. In summary, melatonin may be involved in lipid metabolism by regulating adipogenesis, lipogenesis, and lipolysis in different ways.

genes responsible for lipogenesis, including SREBP1c, FASN, SCD1, ACACA, and PPARγ [204,205]. TG synthesis in subcutaneous adipocytes was downregulated in melatonin-treated obese mice, along with a reduction in the expression of Agpat-2, Lpl, and Dgat2, which are associated with TG biosynthesis [206]. Similarly, melatonin supplementation could limit the lipogenesis pathway to regulate fatty liver syndrome by HFD-induced hyperlipidemia in Syrian hamsters [207]. Likewise, our group reported that melatonin not only significantly inhibited the size of adipocytes but also reduced the mRNA expression of lipogenesis-related genes in epididymal white adipose tissue (Epi-WAT) [42]. Taken together, these findings indicate that melatonin might act as a regulator of anti-lipogenesis, and the mechanisms need to be further elucidated to support related drug development.

Similarly, melatonin is also closely related to lipolysis. Lipolysis is a process in which fat stored in white adipocytes is gradually hydrolyzed by lipases to release free fatty acids and glycerol for oxidative utilization by other tissues or cells. Lipolysis is triggered by various stimuli through hormones, including epinephrine, norepinephrine, glucagon, and insulin. It also requires a variety of enzymes and proteins, such as hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL) and perilipin-1. Studies have shown that melatonin decreases lipolysis and hepatic ketogenesis by reducing the phosphorylation of PKA, HSL, and perilipin-1 in white adipose tissue (WAT) and PKA, AMPK, and acetyl-CoA carboxylase (ACC) in the liver, thereby improving SGLT2i-induced ketoacidosis [208]. Moreover, melatonin might reduce serum lipids in rats treated with combined antiretroviral therapy by inhibiting lipolysis or cholesterol absorption [209]. Whereas it was indicated that melatonin upregulated lipolysis of intramuscular fat (IMF) by stimulating the PKA and ERK1/2 signaling pathways, as well as increasing mitochondrial biogenesis to limit IMF deposition [210]. It is well known that lipolysis activity is closely related to thermogenesis [211]. Melatonin treatment (10 mg/kg/day) for 6 weeks could promote the levels of thermogenic proteins, including UCP1 and PGC-1a, and drive browning of WAT to reduce obesity in Zucker diabetic fatty rats [212]. Additional investigations showed that exogenous melatonin could sympathetically increase lipolysis and browning in WAT to ameliorate obesity in Siberian hamsters [213]. It was also demonstrated in our work that melatonin mediated BAT browning and thermogenesis by regulating the related mRNA expression of marker genes to improve metabolism in HFD-induced obese mice [42]. To put it in a nutshell, these studies suggest that melatonin has different effects in coordinating lipolysis, which might be caused by the inconsistency of experimental samples, treatment time or method, and evaluation indexes, but the intricate role of melatonin has not been explained clearly.

Here, we summarized the possible mechanisms of melatonin mediated signaling pathways in lipid metabolism in Fig. 3. Many studies have shown that there is a close relationship between melatonin and lipid metabolism, but for adipogenesis and lipolysis, their effects on different types of adipose tissue and cells are not the same. Additional experiments are needed to clarify its role in different tissues and cells, as well as the differences in time- and dose-effects, which would lay the foundation for melatonin interventions to improve obesity and related metabolic diseases.

Some evidence supports the use of melatonin as an adjuvant therapy to prevent cancers associated with abnormal lipid metabolism. Interestingly, melatonin reduces the risk of obesity-related breast cancer. Melatonin could reduce body fat mass, improve blood glucose levels and insulin resistance in obese women, as well as inhibit aromatase expression and increase adiponectin secretion, counteracting the risk of breast cancer associated with increased leptin concentration [214]. Additional study indicated that disordered morning circadian rhythms were associated with polycystic ovary syndrome and metabolic disorders in obese girls [215]. Furthermore, melatonin could up-regulate “tumor slimming” and down-regulate clear cell renal cell carcinoma

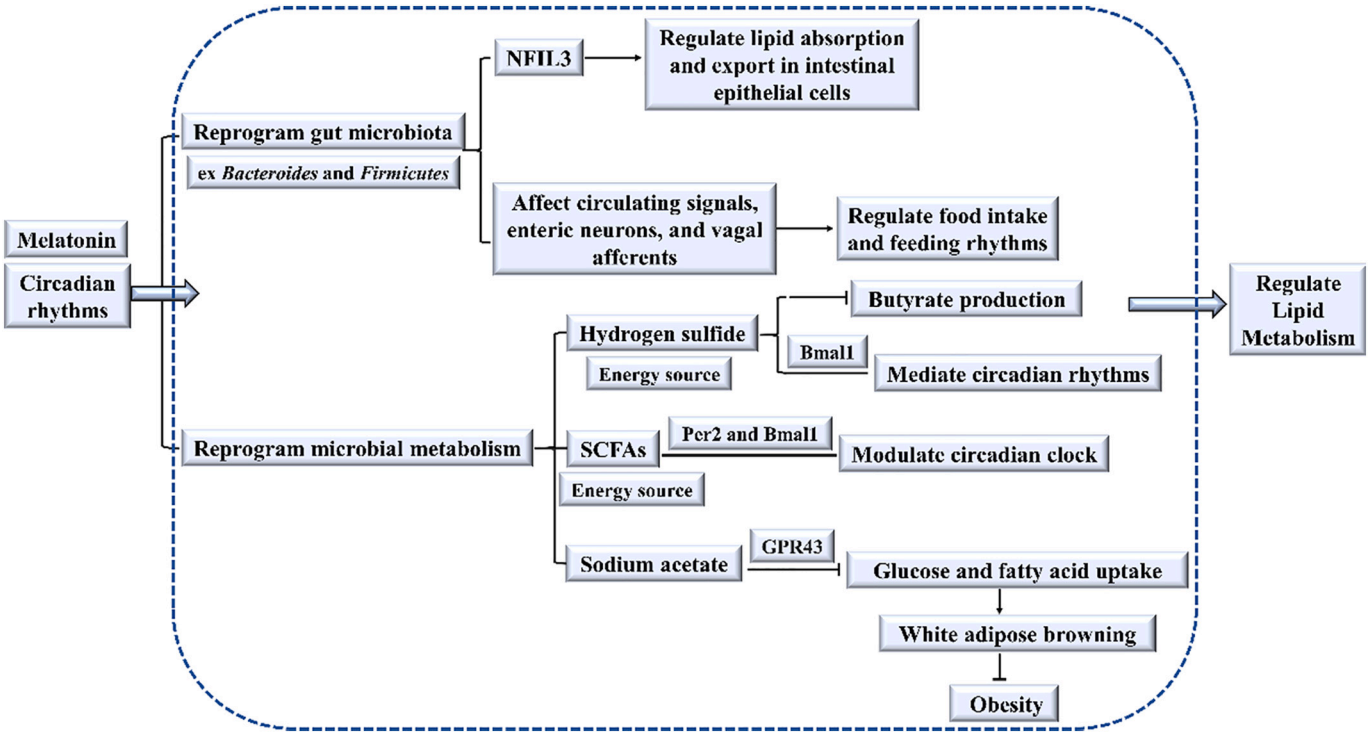

Fig. 4. Schematic representation of potential interactions between circadian rhythms, microbiota, and lipid metabolism. The main role of melatonin is to regulate circadian rhythms. Melatonin regulates lipid homeostasis by the circadian clock and microbiota. The gut microbiota is a potential mechanism of the circadian clock-lipid metabolism interaction. Reprogramming the gut microbiota and microbial metabolism could be used for obesity care.

(ccRCC) progression by PGC1A/UCP1-mediated autophagy and lipid browning [216]. Although compelling experimental evidence has shown that the anti-tumor effect of melatonin can easily reduce the risk of obesity-related cancers, there is still a lack of clinical trials on this topic, especially CRC, which needs to be further explored in future experiments.

5. Melatonin and the gut microbiota

As far as we know, in addition to the pineal gland, the GIT is one of the main sources of melatonin, and its concentration in the GIT is many times higher than that in the pineal gland or plasma [52], suggesting that melatonin has key roles in the gut. The human body contains a large number of microbes, approximately 70% of which are located in the gut [217], and we also know that the dense gut microbiota is an essential component of the gut [218]. These hundreds of billions of bacteria regulate a variety of host physiological or pathological activities to sustain metabolic homeostasis. Recent research has increasingly focused on the relationship between melatonin and the gut microbiota, including circadian rhythms, immunomodulation, lipid metabolism, intestinal health, or other aspects, to elucidate their clinical significance in developing medical value.

Melatonin would maintain homeostasis of the GIT and gut microbiota by regulating circadian rhythms. It is known that the GIT has a circadian rhythm that regulates the expression of clock genes, motility, and secretion in vertebrates [219,220]. Interestingly, recent studies have also shown that the gut microbiota is regulated by host rhythmic expression [221]. To explain how the host's circadian clock regulates the microbiome, Jiffin K. Paulose et al. proved that after isolation from the human intestine, melatonin could specifically increase the number of intestinal bacteria, namely, *Enterobacter aerogenes*. This species is sensitive to melatonin secreted by the gastrointestinal lumen and shows a specific rhythm of swarming and motility, which suggests that the microbiome could achieve interactions between symbiotic bacteria and intestinal tissues through possible signaling pathways, such as the signal of rhythmic melatonin levels, to maintain its homeostasis [222].

Furthermore, melatonin would mediate gut microbiota to regulate gut-brain axis. The gut-brain axis is a bidirectional information communication system that integrates the functions of the brain and the intestine. The bidirectional interaction between the central nervous system, the intestinal nervous system and GIT has also been paid more and more attention. Changes in the function of the gut-brain axis are involved in the occurrence of a variety of gastrointestinal diseases, such as IBD and related functional gastrointestinal diseases. The gut microbiota is a key element of this axis, which performs its functions not only locally but also on multiple levels. Intestinal bacterial metabolites can be absorbed into the blood and transported through the blood-brain barrier into the cerebrospinal fluid to regulate brain function. Disturbances of host circadian rhythms are an independent risk factor for CRC. Melatonin is a key hormone in circadian rhythmicity. It seems that it could improve the circadian rhythm, and have an impact on the metabolism of the local gut microbiota and modulate changes in their composition to alleviate microbial dysbiosis. In addition, gut microbiota could regulate the tight junctions between epithelial cells, thereby reducing intestinal permeability and protecting the intestinal barrier. Interestingly, melatonin appears to inhibit the entry of bacteria and their harmful metabolites into mesenteric lymphatic tissue, regulate inflammatory immune reactions and affect vagus and spinal afferent nerves. Moreover, melatonin can also regulate enteroendocrine cells in an autocrine or paracrine fashion to affect the activities of the central nervous system, and inhibit the process of CRC. There is growing evidence supporting bidirectional communication between circadian rhythms and the gut microbiota. However, it is no doubt that the precise underpinnings of the mechanisms involved remain unclear. More work is needed to study how the microbiota-gut-brain axis and melatonin influence CRC, and how they interact with one another in the context of CRC in the future.

A key precursor of melatonin, 5-HT is regarded as a molecular signal factor in the brain and enteric nervous system. 5-HT regulates functions

like secretion, vasodilation, peristalsis and intestinal function. Changes in 5-HT occur in the microbiota-gut-brain axis disorders. The spore-forming bacteria from the gut microbiota of mice can promote the 5-HT biosynthesis of colonic enterochromaffin cells and regulate the 5-HT concentration in the colon and blood. The gut microbiota promotes the production and homeostasis of intestinal 5-HT through short-chain fatty acids. The production and composition of intestinal mucus and the development of enteroendocrine cells secreting 5-HT are also affected by the gut microbiota. The increasing evidence supports the dual role of 5-HT in protecting intestinal homeostasis and accelerating colon carcinogenesis [223], due in part to altered 5-HT pathway, mutations, and inflammation [224]. However, studies have demonstrated protective role of melatonin in CRC. Although 5-HT, as a precursor for melatonin, has multiple effects through its many receptors, it is important to note that the regulation of melatonin, such as the uptake of 5-HT transporters, would have significant impact on the levels of melatonin synthesis. More work needs to be done to better understand the modulation of the 5-HT pathway and help design novel anti-cancer therapies. Moreover, current findings indicate that the gut microbiota can produce short-chain fatty acids (such as acetic acid, propionic acid, and butyric acid), as well as catecholamines, serotonin, melatonin, and other signaling factors that regulate host intestinal endocrine secretion, acting on the nervous and immune systems, and interacting with the host [225]. The gut microbiota acts on the brain by regulating the secretion of hormones such as brain-intestinal peptides from intestinal endocrine cells to achieve information communication between the brain and the intestine. Importantly, a variety of potential mediators have been linked to melatonin, the gut microbiota, and gut-brain axis, but the exact mechanisms of these connections have yet to be elucidated.

Combined with recent studies, we found that the gut microbiota and melatonin metabolism may interact to regulate lipid homeostasis. Our group has reported that melatonin significantly reduced the number of OTUs and the richness and diversity of gut microbiota in HFD-induced obese mice by analyzing the gut microbiota composition. Meanwhile, LEfSe analysis revealed that melatonin significantly increased the relative abundance of *Akkermansia*, which is a kind of beneficial bacterium, to reduce weight in obese subjects. In addition, melatonin significantly decreased the relative abundance of *Alistipes*, *Anaerotruncus*, and *Helicobacter marmotae*, which are harmful bacteria, to prevent obesity [42]. In short, melatonin was able to normalize the gut microbiota of obese mice, thereby inhibiting weight gain. Consistent with our group's observations, in a mouse model of light rhythm disorders, *Anaerotruncus*, *Alloprevotella*, and *Faecalibaculum* presented a decreasing abundance upon supplementation with melatonin, which, as previously described, tended to accumulate in obese mice. Thus, these data indicate that melatonin treatment has shown beneficial effects in improving intestinal microecological disorders induced by exposure to constant light [44]. Additionally, the results of another compelling study indicated that microbiota transplantation from HFD-fed mice aggravated lipid disorders in obese mice; however, intervention of the fecal microbiota in the melatonin-treated group relieved the lipid dysmetabolism, suggesting that the gut microbiota are involved in the melatonin to lipid metabolism induced by HFD, and its possible mechanism may be related to reprogramming the gut microbiota to reverse lipid metabolism [43]. In short, the primary role of melatonin is to regulate circadian rhythms. Some studies have reported that melatonin regulates lipid homeostasis by the circadian clock and microbiota. Fig. 4 illustrates the potential interactions of circadian rhythms, microbiota, and lipid metabolism.

Certainly, the interaction of melatonin with the gut microbiota is also reflected in other metabolic activities under special conditions regulating intestinal health. Weaning leads to a decrease in the intestinal microbial diversity, particularly in *Lactobacillus*, and an increase in *Clostridium*, *Prevotella*, and *facultative anaerobes*, including *E. coli*. However, melatonin could effectively alleviate weaning stress, increase the daily weight gain of animals after weaning; improve the intestinal morphology, including villus length, crypt depth, and villus to crypt ratio; and regulate the composition and metabolism of the gut microbiota. The above data clarified the mechanism of melatonin-mediated gut microbiota changes to relieve weaning stress, providing a target for intestinal infection and regulation of weaning stress in piglets and is of great significance for promoting intestinal health and improving the productivity of piglets [226].

In addition, in the sleep-deprived mouse model, colonic mucosal damage was characterized by decreased goblet cells and PCNA-positive cells, as well as reduced expression of associated marker factors, such as MUC2 and tight junction proteins. Meanwhile, the diversity and abundance of colonic microbiota were limited. Among them, beneficial bacteria, including *Akkermansia*, *Bacteroides*, and *Faecalibacterium*, were reduced, while pathogenic bacteria such as *Aeromonas* were significantly increased. However, melatonin supplementation could reverse these changes caused by sleep deprivation, which suggested that melatonin could regulate the gut microbiota to improve the effects of sleep deprivation on intestinal barrier dysfunction [227]. Interestingly, in the DSS-induced colitis mice, compared with the DSS group, melatonin treatment increased the richness of *Coprococcus* and *Ruminococcaceae*, suggesting that melatonin could improve the ability of antioxidative stress in colitis mice and regulate the gut microbiota, thus improving intestinal health [228]. Meaningfully, our recent research has investigated the effects of melatonin treatment on the intestinal morphology of mice with constant light-induced rhythm disturbances and found that melatonin could ameliorate rhythm disorders and intestinal dysfunction [44]. These studies indicated that melatonin mediates gut microbiota changes to regulate intestinal health and homeostasis, but the specific mechanism remains to be further explored.

Additional studies have expanded the interaction mechanism between melatonin and the gut microbiota, and they showed that melatonin-mediated gut microbiota changes play a pivotal role in such aspects as goose productive performance [229], spinal cord injury in mice [230], and muscle exercise in rodents and humans [231]. In summary, ample studies have shown that melatonin has remarkable interactions with the gut microbiota and that melatonin is affected by a variety of comprehensive factors, such as experimental species, diet, hunger, age, jet lag, and the external environment, such that the interaction between melatonin and the gut microbiota may vary according to different conditions. Therefore, the signaling pathways or molecules that connect melatonin to clusters need to be further explored to supplement the potential mechanism of melatonin in cooperation with the gut microbiota in regulating physiological activities.

6. Conclusion

Melatonin is a neuroendocrine hormone secreted mainly by the pineal gland, GIT, retina, testes, and lymphocytes. Its physiological functions include adjusting the circadian clock, inducing sleep, regulating the endocrine system, regulating lipid homeostasis, enhancing immunity, and anti-cancer effects. Growing evidence has focused on a possible benefit of melatonin on CRC. Importantly, one of the most ideal anticancer roles of melatonin is to regulate the homeostasis of the tumor microenvironment. Current studies suggest that the gut microbiota and inflammation in the tumor microenvironment play an important role in the development and treatment of CRC. When the gut microbiota is disordered, the interaction between special bacteria (such as *Bacteroides fragilis* and *Fusobacterium nucleatum*) could lead to a series of inflammatory responses in colorectal epithelial cells. A long-term inflammatory state could also cause cancer development in colorectal epithelial cells, ultimately promoting the carcinogenesis of CRC. HFD- or obesity-induced microbiota imbalance increases the risk of pathogen infection and promotes intestinal inflammation, thereby inducing intestinal tumors. The ability of the microbiome to interact with the colonic epithelium can provide a useful insight into the relationship between obesity and increased CRC risk. The possible mechanism of melatonin is to first bind to several specific receptors, and then stimulate signal

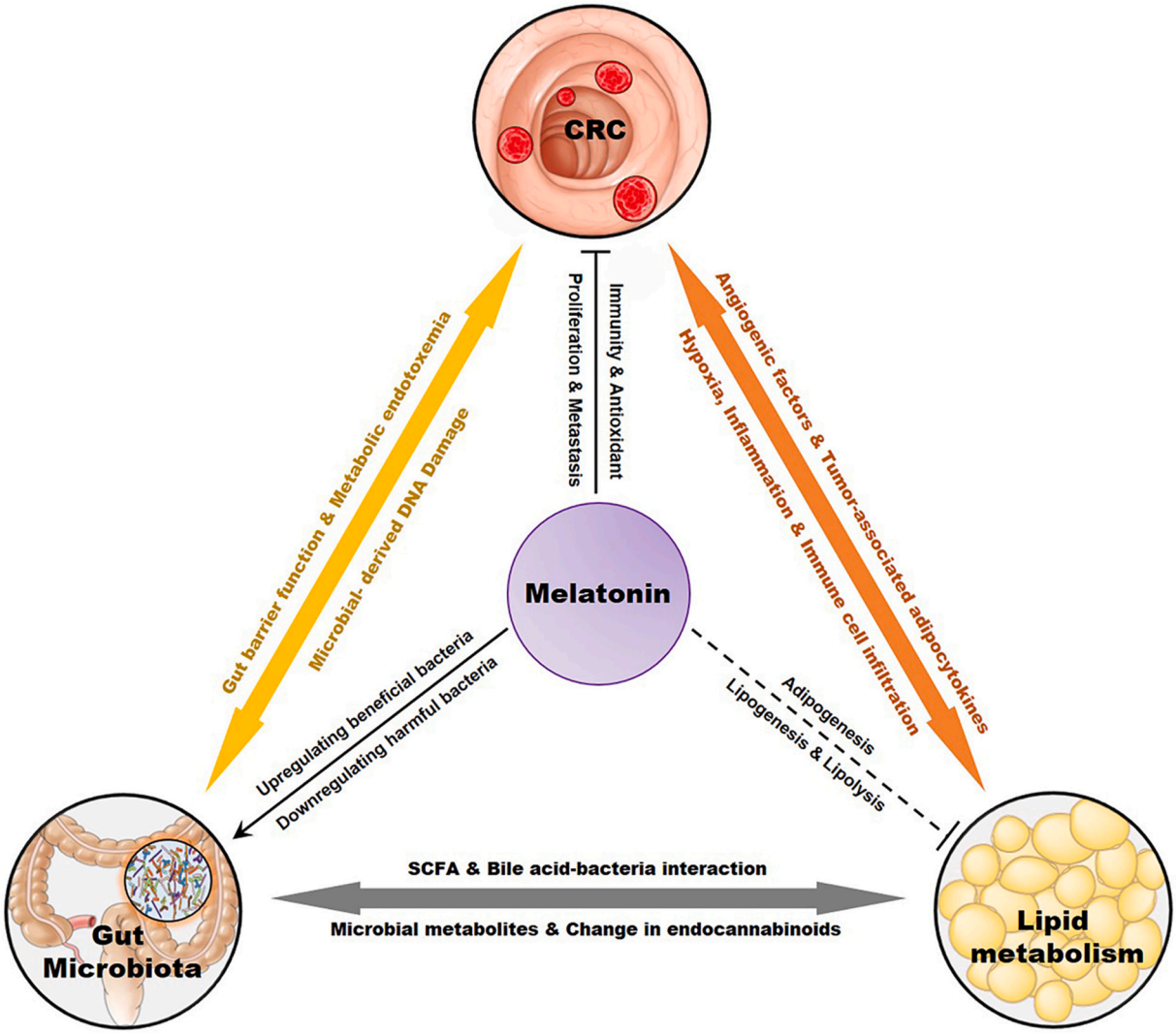

Fig. 5. Schematic representation of melatonin linking lipid metabolism and gut microbiota in CRC. Obesity and dysbacteriosis have been increasingly regarded as the main risk factors of CRC. With mutual impact on each other, they tend to form a vicious circle under certain conditions. Interestingly, melatonin plays a pivotal role as a bridge in the above-mentioned cycle through regulating multiple signaling pathways. There may be an inevitable association among melatonin, intestinal microbiome, lipid metabolism, and CRC, which promotes the positive role of melatonin in inhibiting the development of CRC.

transduction mediated by different transcription factors to produce responses. The eventual outcome is that while directly affecting gene expression, it also regulates the initiation and progression of carcinogenic pathways. It would be seen that melatonin not only mediates cell signaling to play multiple roles such as coordinating the gut microbiota, it also has direct anti-inflammatory, immune regulation and antioxidant activity. Moreover, it also reduces angiogenesis, thereby limiting the amount of energy and oxygen absorbed by the tumor [232,233]. Altogether, melatonin participates in the homeostasis of the tumor microenvironment through various pathways and mechanisms to regulate tumor progression. We summarized the relationship of melatonin linking lipid metabolism and the gut microbiota in CRC (Fig. 5).

The exact mechanisms need to be further elucidated: first, maleficent microbiota is one of the initial contributors to CRC. How changes in gut microbiota distribution and richness lead to inflammation and immune responses and cause malignant changes in intestinal mucosal cells needs additional study. How the gut microbiota specifically mediates obesity-related CRC in different conditions also needs additional study. In addition, how melatonin, in conjunction with various beneficial metabolites of the gut microbiota, directly or indirectly inhibits the development and progression of CRC deserves further attention. Finally, whether melatonin could reshape intestinal homeostasis to prevent tumorigenesis of CRC, especially in obesity-related CRC, is still an open question.

Taken together, the gut microbiota and lipid metabolism are two potential targets in the therapeutic applications of melatonin in obesity associated-colorectal cancer. The gut microbiota and melatonin may interact to regulate lipid metabolism and intestinal physiological functions. Due to the complexity of the gut microbiota, the mechanism of how microbes mediate intestinal sensing of melatonin signals and their feedback to regulate lipid metabolism intestinal physiological functions and CRC needs further study. More epidemiological, observational, and experimental research is required to promote the better application of melatonin to solve the problem of increasing incidence of CRC.

Funding

This research was funded by the National Natural Science Foundation of China (NO. 31571164 and NO. 82070901). This research also supported by BNU Interdisciplinary Research Foundation for the First-Year Doctoral Candidates (Grant NO. BNUXKJC1924).

Declaration of competing interest

The authors declare that there is no conflict of interest.

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