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Role of HCA_2 in Regulating Intestinal Homeostasis and Suppressing Colon Carcinogenesis

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Hydroxycarboxylic acid receptor 2 (HCA_2) is vital for sensing intermediates of metabolism, including β-hydroxybutyrate and butyrate. It also regulates profound anti-inflammatory effects in various tissues, indicating that HCA_2 may serve as an essential therapeutic target for mediating inflammation-associated diseases. Butyrate and niacin, endogenous and exogenous ligands of HCA_2, have been reported to play an essential role in maintaining intestinal homeostasis. HCA_2, predominantly expressed in diverse immune cells, is also present in intestinal epithelial cells (IECs), where it regulates the intricate communication network between diet, microbiota, and immune cells. This review summarizes the physiological role of HCA_2 in intestinal homeostasis and its pathological role in intestinal inflammation and cancer.

Keywords: HCA_2, intestinal homeostasis, anti-inflammatory, intestinal inflammation, colon cancer, mucosal immunity, microbiota

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INTRODUCTION

The intestinal tract is an organ system with specialized architecture that functions to digest food, and extract and absorb energy and nutrients. It also secretes over 20 different hormones and harbors more than 640 different species of bacteria (1). Physiological and pathophysiological events that trigger the breakdown of intestinal homeostasis negatively impact intestinal health, and may result in intestinal disorders including inflammatory bowel disease (IBD) and colitis-associated cancer. IBD is a chronic and life-threating disease characterized by prolonged inflammation of the digestive tract (2, 3). IBD encompasses two conditions, Crohn's disease and ulcerative colitis. Crohn's disease can affect any part and layer of the gastrointestinal tract, while ulcerative colitis is usually limited to the innermost layer of the colon and rectum (4). Both Crohn's disease and ulcerative colitis are characterized by episodes of fatigue, abdominal cramping, rectal bleeding, diarrhea, weight loss, and the influx of immune cells that produce cytokines, proteolytic enzymes, and free radicals (5, 6). Patients with IBD are at increased risk of developing colitis-associated cancer which is difficult to treat and has high mortality (>50%) (7, 8). In 2015, an estimated 1.3% of US adults reported living with IBD, with cases increasing worldwide (9, 10). The global spread of IBD is associated with the host genetic background, intestinal microbiota, diets, environments and immunological dysregulation (4, 11, 12).

The intestinal tract represents the largest compartment of the immune system in the body (13), with intestinal health implicated in controlling disease development not only within itself but also throughout the body. To maintain intestinal homeostasis, a multi-pronged approach including the immune system, microbial ecosystem and diet is necessary. A versatile receptor, hydroxycarboxylic acid receptor 2 (HCA_2), is capable of both nutrient sensing and immunomodulation, lending to its popularity as a potential target for the promotion of intestine health.

In 1993, HCA_2 was identified as an orphan receptor (GPR109A) (14, 15), and later described in mice as a "protein upregulated in macrophages by interferon-gamma (IFN-γ)" (PUMA-G) (16). In 2003, several studies reported that HCA_2 is a receptor for niacin and functions to mediate its antilipolytic effects in adipocytes (17–19). Benyó et al. and Hanson et al. subsequently demonstrated that binding of niacin to HCA_2 on Langerhans cells and keratinocytes is also responsible for the niacin-induced cutaneous flushing reaction, involving release of prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2) (20, 21). In 2005, the ketone body β-hydroxybutyrate (β-OHB) was identified as an endogenous ligand of HCA_2 (22). This resulted in the deorphanization of the receptor, which was subsequently renamed hydroxycarboxylic acid receptor 2 (HCA_2) (23). Most recently, butyrate, a short-chain fatty acid (SCFA) bacterial product in the colon lumen generated at high concentrations (10–20 mM) from dietary fiber fermentation, was recognized as an endogenous ligand of HCA_2 (24). Butyrate activation of HCA_2 plays an important role in the maintenance of intestinal homeostasis (24). New synthetic ligands of HCA_2 have been developed, such as acipimox, GSK256073 and derivatives of pyrazole-3-carboxylic acid or cyclopentapyrazole (25–27).

HCA_2 is widely expressed in various tissues and cell types, including adipose tissue, spleen, lung, lymph node and intestine. HCA_2 is predominantly expressed not only in both white and brown adipocytes, but also in diverse immune cells, including dendritic cells (DCs), monocytes, macrophages, neutrophils and epidermal Langerhans cells, but not lymphocytes (16, 18, 21, 28, 29). Interestingly, several cytokines show the ability to regulate the expression of HCA_2 in immune cells. HCA_2 expression is upregulated in macrophages and monocytes after IFN-γ treatment (16), and the expression of HCA_2 in macrophages is significantly increased by proinflammatory stimulants lipopolysaccharide (LPS), interleukin (IL)-6 and IL-1β (30). Colony-stimulating factor 2 (CSF2) increases HCA_2 expression level in neutrophils (29). HCA_2 is also present in intestinal epithelial cells (IECs), retinal pigment epithelium, hepatocytes, keratinocytes and microglia (21, 31–34). Notably, both mRNA and protein levels of HCA_2 in IECs are drastically reduced in germ-free mice compared to conventional mice, due to the absence of gut bacteria. These changes are reversed when the intestinal tract of germ-free mice is re-colonized (35).

While the anti-lipolytic effects of HCA_2 are well-known, more recent studies have demonstrated that activation of HCA_2 by endogenous and exogenous ligands is associated with anti-inflammatory effects in numerous disease states (25, 31, 36–41). Early studies showed that activation of HCA_2 in various cell types could trigger different downstream signaling events and effects (26) (**Figures 1A–F**). In adipocytes, activation of HCA_2 inhibits lipolysis (18, 42, 43) (**Figure 1A**). In hepatocytes, HCA_2 mediates hepatic *de novo* lipogenesis and decreases lipid accumulation in liver (44, 45) (**Figure 1B**). In IECs, ligand binding to HCA_2 activates NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, which promotes the maturation of IL-18 for secretion (46, 47) (**Figure 1C**). IL-18 is a critical effector molecule in intestinal disorders and is required for IEC proliferation (48). HCA_2 also suppresses basal and LPS-induced nuclear factor-kappa B (NF-κB) activation in normal and cancer colonocytes (24) (**Figure 1C**). In retinal pigment epithelium, HCA_2 exerts dual effects depending on the concentration of the agonist. 4-hydroxynonenal, an HCA_2 agonist, can induce either an anti-inflammatory response or apoptosis (49) (**Figure 1D**). In Langerhans cells, HCA_2 causes cutaneous flushing reaction (20, 21) (**Figure 1E**). In macrophages, activation of HCA_2 exerts an anti-inflammatory effect (50, 51) (**Figure 1F**). HCA_2 also represses chemokine-induced migration of macrophages (30) (**Figure 1F**). HCA_2 shows anti-inflammatory effects in microglia and human monocytes (52–54) (**Figure 1F**). In DCs, HCA_2 activation decreases IL-6 levels and increases IL-10 levels and upregulates expression of RALDH1, which synthesizes retinoic acid (RA) from retinol. RA is necessary for promoting regulatory T cells (Tregs) function and proliferation, especially in the gut in both murine and human DCs (55–57) (**Figure 1F**). In neutrophils, niacin-mediated HCA_2 activation increases Bcl-2 associated agonist of cell death (BAD) levels, a pro-apoptotic member of the Bcl-2 family (58) (**Figure 1F**). Collectively, these studies clearly demonstrate that HCA_2 plays a critical role in nutrient sensing and host protection against pro-inflammatory insults in multiple cell types using various signaling mechanisms.

INTESTINAL HOMEOSTASIS AND HCA_2: IMMUNE CELLS, INTESTINAL EPITHELIUM, MICROBIOME, AND METABOLITES

The intestinal tract, comprised of small intestine, large intestine/colon, and rectum, is the central location for nutrient and water absorption. It harbors more than 10^{13} microorganisms, contains over 20 different hormones, and serves as the single largest immune compartment in the body (13, 59). Consequently, building and maintaining a homeostatic intestinal tract is a highly complex and broad concept that encompasses a multi-disciplinary approach including the immune system, host cells, gut microbiota and nutrients. Further complexity arises from the mutual interactions between the intestinal tract and other organ systems.

The intestinal mucosa, a crucial site of innate and adaptive immune regulation, is comprised of IECs, lamina propria and muscularis mucosa (**Figure 2**). IECs are specialized epithelia comprised of many different cell types: epithelial stem cells which continuously self-renew by dividing and generate all differentiated intestinal cell types, enterocytes which absorb water and nutrients, goblet cells which secrete mucins to form a mucus layer boundary between the gut microbiota and host tissue, Paneth cells which secrete anti-microbial peptides, enteroendocrine cells which secrete hormones and cytokines capable of systemic or local effects, and microfold cells (M cells) which connect to the intestinal lymphoid follicles (60–63). The intestinal epithelium is bound together by tight junction proteins, which regulate the paracellular permeability and are essential for the integrity of the epithelial barrier. Tight junction proteins prevent harmful substances such as LPS, foreign antigens, toxins and microorganisms from entering into the blood stream (64).

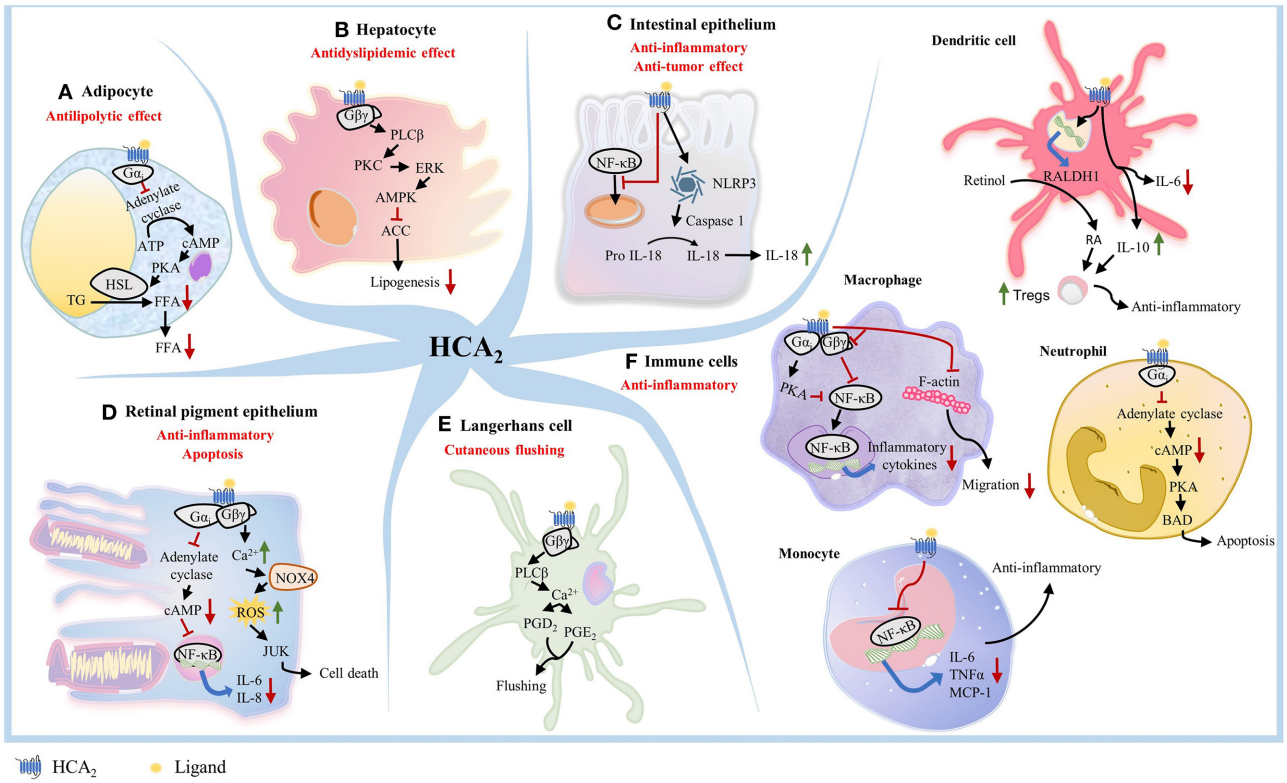

FIGURE 1 | HCA_2 triggers different downstream signaling pathway in different cell types. **(A)** In adipocytes, activation of HCA_2 triggers a Gαi-mediated inhibition of adenylate cyclase activity, which leads to lower intracellular cAMP levels, reduced protein kinase A (PKA) activity, and further reduces the activity of hormone sensitive lipase (HSL), an important lipolytic enzyme. This inhibition of lipolysis results in a decreased release of free fatty acids into the circulation. **(B)** In hepatocytes, activation of HCA_2 mediates the protein kinase C (PKC)-extracellular signal-regulated kinase (ERK) signaling pathway, leading to phosphorylation of AMP-activated protein kinase (AMPK) and inhibition of acetyl CoA carboxylase (ACC). This results in an inhibition of hepatic *de novo* lipogenesis and a remarkable decrease of lipid accumulation in liver. **(C)** In colonocytes, ligand binding to HCA_2 suppresses NF-κB activation and activates NLRP3 inflammasome, which recruits caspase-1 and promotes the maturation of IL-18 for secretion. **(D)** In retinal pigment epithelium, HCA_2 exerts either an anti-inflammatory response through the Gαi/cAMP/NF-κB pathway, or apoptosis through the $G\beta\gamma/Ca^{2+}$/NOX4/ROS/JNK pathway. **(E)** Within Langerhans cells, HCA_2-mediated $G\alpha_i$ activation primarily results in the Gβγ-complex released from activated $G\alpha_i$, thereby increasing intracellular calcium concentration by mobilizing Ca^{2+} release from the endoplasmic reticulum and ultimately driving the formation of PGD_2 and PGE_2, which are released to the dermal layer and cause cutaneous flushing reaction. **(F)** In macrophages, activation of HCA_2 involves inhibition of NF-κB, thereby exerting an anti-inflammatory effec. HCA_2 activation in macrophages also represses F-actin and blocks Gβγ signaling to inhibit chemokine-induced migration of macrophages. HCA_2 also shows anti-inflammatory effects in Parkinson's disease models by inhibiting the phosphorylation of the NF-κB p65 signaling pathway in microglia. HCA_2 suppresses LPS-induced NF-κB activation in human monocytes, resulting in decreased transcription of IL-6, TNFα, and MCP-1. In DCs, HCA_2 activation decreases IL-6 level and increases IL-10 level, also upregulates expression of RALDH1, which synthesizes RA from retinol. RA and IL-10 promote Treg cell proliferation. In neutrophils, niacin-mediated HCA_2 activation inhibits PKA activity and subsequently increase BAD levels, which drives apoptosis of neutrophils.

IECs are well-equipped to recognize luminal pathogens by expressing different pattern recognition receptors, including NOD-like receptors (NLRs) in the cytosol and Toll-like receptors (TLRs) on the apical membrane and in endosomes, with the capacity to sample gram-positive and gram-negative infectious bacteria (65, 66). Additionally, various immune cells, including intraepithelial γδ-T cells and specialized mucosal macrophages, reside intercalated in the IEC layer, and function to sample pathogens from the lumen (67). IECs also express multimeric protein complexes known as inflammasomes that are important for intestinal immune homeostasis, inflammation, and tumorigenesis. Ligand stimulation of HCA_2 expression is associated with increased NLRP3 inflammasome activation, which processes the proIL-18 into IL-18, an anti-inflammatory cytokine which is critical in regulating mucosal immunity and epithelial integrity (46) (**Figure 2**). Recent studies demonstrate that mice deficient in IL-18 have increased pathogenesis of colitis and colon cancer, and dysregulation of IL-1β expression exacerbates IBD (48, 68).

Immune cells are found in intestinal epithelium (intraepithelial lymphocytes) as well as in organized lymphoid tissues/organs, such as the Peyer's Patches (PPs) and mesenteric lymph nodes (MLNs). Substantial amounts of scattered innate and adaptive effector immune cells are also widely distributed in the lamina propria, which is a loosely packed connective tissue layer underlying the IEC layer (69–71) (**Figure 2**). Collectively, the lamina propria and IECs form

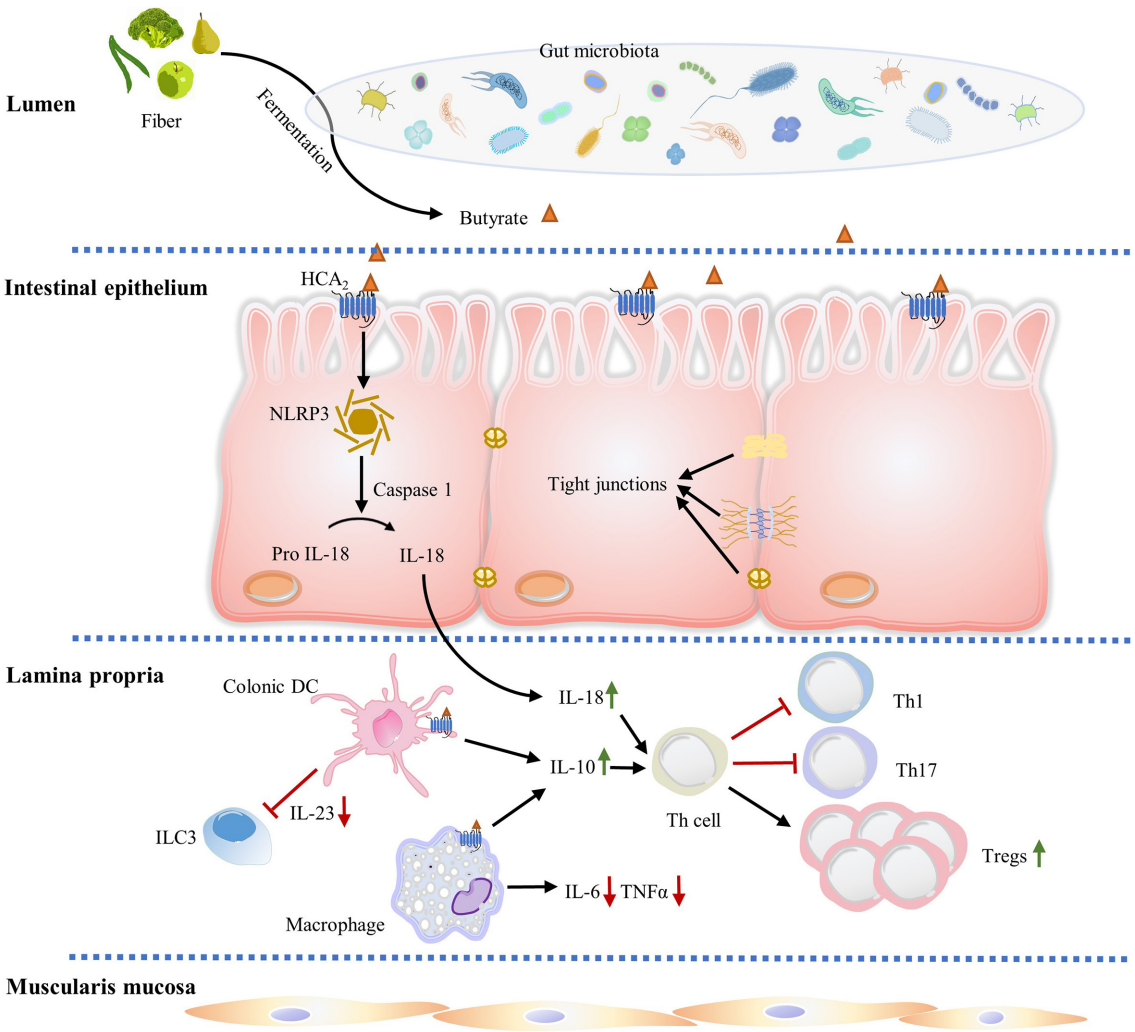

FIGURE 2 | HCA_2 regulates gut immune homeostasis. Butyrate produced by gut microbiota fermentation activates HCA_2 expressed on IECs, macrophages, and dendritic cells. In IECs, HCA_2 stimulation is associated with increased inflammasome activation, which processes pro-IL-18 into mature IL-18, which is critical in regulating mucosal immunity and epithelial integrity. IL-18 and ligand induced anti-inflammatory IL-10 (from intestinal macrophages and dendritic cells) promote naïve T cells differentiation and proliferation into immunomodulator Treg cells, which protect intestine from inflammation and colitis associated cancer. IL-18 and IL-10 also decrease the proinflammatory Th1 and Th17 cell number. In addition, HCA_2 decreases proinflammatory cytokine IL-6 and TNF-α expression in intestinal macrophages and inhibits dendritic cell-induced IL-23 production to suppress ILC3-associated colonic inflammation.

a unique immunological compartment which contains the largest population of immune cells in the body, as well as supply the nerve, blood and lymph drainage for the entire mucosa (71). The lamina propria contains lymphocytes and numerous innate immune system-related cell populations, including eosinophils, macrophages, DCs, immunoglobulin (Ig) A secreting plasma cells, mast cells and innate lymphoid cells (ILCs) (71–73) (**Figure 2**). ILCs are a family of three innate effector cells (ILC1, ILC2, and ILC3) that are critical modulators of mucosal immunity (74). Particularly, ILC3 is implicated in innate intestinal inflammation though production of IFN-γ, IL-17, and IL-22 under induction by IL-1β and IL-23 (75). Depletion of ILC3 abrogates innate colitis, suggesting ILC3 is responsible for the intestinal pathogenesis (75). Bhatt et al. showed that HCA_2 signaling limits IL-23 production by DCs, which further suppresses ILC3-mediated colonic inflammation (76) (**Figure 2**). Activation of HCA_2 expressed on immune cells in colon lamina propria also modulates the frequency and number of Treg cells and IL-10 producing T cells (34) (**Figure 2**).

The gut microbiota is considered a commensal metabolic organ with critical roles in energy salvaging and nutrient absorption. It also functions in systemic immunity regulation and protection of the colonized host by eliminating pathogenic bacteria (77). Tan et al. compared fecal microbiota composition between WT and $HCA_2^{-/-}$ mice fed a high-fiber diet, and determined that loss of HCA_2 alters microbiota composition dramatically (57). Specifically, $HCA_2^{-/-}$ mice show an increase

of *Verrucomicrobiae*, *Alphaproteobacteria*, and *Bacilli*, and a decrease of *Bacteroidia* (57). Germ-free animals show extensive impaired maturation of isolated lymphoid follicles, PPs and MLNs, and are also defective in antibody production and cytokine secretion compared to conventional animals (78). The status of germ-free animals converts after colonization with normal gut microbiota, suggesting a dynamic relationship between the commensal organism and host immune system. Gut microbiota also plays an irreplaceable role in the regulation of host intestinal gene expression with around 700 genes altered remarkably in mice under germ-free conditions. Among them, the expression of *Hca*$_2$ is reduced significantly in the ileum and colon under germ-free conditions, which is restored to normal levels after introduction of gut bacteria (35).

When the balance of gut microbiota ecosystems is disturbed (dysbiosis), tight junction barrier is compromised. Antigens, toxins and microorganisms can pass through the epithelium and trigger the immune response. Intestinal dysbiosis is commonly associated with a series of intestinal and extra-intestinal pathological disorders, including obesity, diabetes mellitus, multisystem organ failure, allergy, asthma, colitis-associated cancer and IBD (77, 79). Specifically, IBD patients shift their gut microbiota composition to an enrichment of *Desulfovibrio, Enterobacteriaceae*, *Ruminococcus gnavus*, and depletion of *Akkermansia Faecalibacterium prausnitzii*, and *Lachnospiraceae* (80).

Multiple evidence suggests that the composition of the intestinal microbiota can be altered by diet within hours to days, leading to aberrant immune responses (81–83). Extensive studies have demonstrated that the structure and function of the gut microbiota rapidly shifts and intestinal atrophy and low-grade inflammation occur under Western diets conditions within 1 day (84–89). Nevertheless, this influence is largely eliminated by manipulating the dietary fiber content in Western diets, allowing for protection against microbiota depletion, amelioration of the inflammation and restoration of colon length (84, 88). These beneficial aspects of fiber are largely attributed to bacterial fermentation products (SCFAs), including acetate, propionate and butyrate. SCFAs are sensed by specific immunomodulating receptors, including HCA_2, GPR41, and GPR43, which are involved in intestinal immunoactivity, intestinal motility regulation and cytokine secretion (90).

Among the SCFAs, butyrate/HCA_2-mediated signaling has received the most attention for its effects on intestinal homeostasis and may provide an important molecular link between gut bacteria and the host (91–94). Numerous studies have confirmed that antibiotic treatment causes gut microbiota dysbiosis by perturbing intestinal immune regulation, evidenced by a reduction in Treg cell numbers within the colon (95, 96). Niacin and HCA_2 agonist supplementation efficiently rescues Treg cell depletion in antibiotic-treated WT mice, but this effect is nullified in $HCA_2^{-/-}$ mice (34). $HCA_2^{-/-}$ mice also show an inflammatory intestinal phenotype and enhanced susceptibility to azoxymethane (AOM) + dextran sulfate sodium (DSS)-induced colitis-associated colon cancer (34). Clinically, patients with ulcerative colitis and colitis-associated cancer suffer a remarkable depletion in the total amount of butyrate-producing bacteria in colon (97, 98), while irrigating the colon with butyrate significantly suppresses intestinal inflammation during ulcerative colitis (99). Hence, HCA_2 is a critical link in the network of diet, microbiota, immune cells, and host cells which are necessary for the maintenance of intestinal homeostasis.

ROLE OF HCA_2 IN INTESTINAL INFLAMMATION

The role of HCA_2 in regulating intestinal immunological response and inflammation is multifaceted. Singh et al. found that the colons of mice lacking HCA_2 present a unique status of $CD4^+$ T cells, also known as T helper cells (Th cells) (34). These cells play an important role in immune regulation, where they mediate the activation of other immune cells though the release of various cytokines. Among the $CD4^+$ T cells, Tregs express the transcription factor Forkhead box protein P3 (Foxp3), which is capable of potently suppressing immune responses. In colonic lamina propria of $HCA_2^{-/-}$ mice, the amount of $Foxp3^+$/Treg cells among $CD4^+$ T cells and anti-inflammatory IL-10 producing $CD4^+$ T cells is significantly less than WT mice, while the frequency and number of $CD4^+$ T cells producing the inflammatory cytokine IL-17 are increased (34). In contrast, a similar fraction of those cells distribute in splenic T cells from both WT and $HCA_2^{-/-}$ mice, suggesting that only the colon $CD4^+$ T cells are specifically influenced by a lack of $HCA_2^{-/-}$ (34). Singh et al. reasoned that this proinflammatory phenotype of the $HCA_2^{-/-}$ mice colon is dependent on colonic DCs and macrophages, since they both express HCA_2 and are critical inducers of naive T cell differentiation (100, 101). They addressed this by testing the ability of colonic DCs and macrophages from both WT and $HCA_2^{-/-}$ mice to induce differentiation of naive $CD4^+$ T cells. As expected, $HCA_2^{-/-}$ colonic DCs and macrophages are defective in expression of retinaldehyde dehydrogenase 1 (RALDH1) and immunosuppressive cytokine IL-10, and express more proinflammatory cytokine IL-6 compared to WT DCs and macrophages. This change in expression leads naive $CD4^+$ T cells to differentiate into proinflammatory Th17 cells, but not Treg cells and IL-10-producing $CD4^+$ T cells (34). Likewise, $HCA_2^{-/-}$ is necessary to maintain normal anti-inflammatory IL-18 levels, as both mRNA and protein expression of IL-18 are significantly decreased in IECs of $HCA_2^{-/-}$ mice (34). Consistent with this evidence, Singh et al. also demonstrated that niacin treatment restored colonic Treg cell numbers in antibiotic-treated WT mice, and butyrate and niacin induced IL-10 and RALDH1 expression and promoted naïve T cells differentiation into Treg cells in macrophages and DCs in an HCA_2-dependent manner (34) (**Figure 2**). In addition, butyrate and niacin increased expression of IL-18 in colonic epithelium of WT mice but not $HCA_2^{-/-}$ mice (34).

Bhatt et al. recently described another anti-inflammatory effect of HCA_2 in restraining microbiota-induced IL-23

production to suppress ILC3-associated colonic inflammation (76). To diminish the influence of the adaptive immune system, they bred $HCA_2^{-/-}$ mice with recombination activating gene 1 (RAG1) deficient mice [no mature B and T lymphocytes (102)] to generate $HCA_2^{-/-}$ $Rag1^{-/-}$ mice. $HCA_2^{-/-}$ $Rag1^{-/-}$ mice spontaneously develop rectal prolapse and exhibit immune cell infiltration of the intestinal lamina propria, which is not seen in $Rag1^{-/-}$ mice under the same conditions (76). In addition, colons of $HCA_2^{-/-}$ $Rag1^{-/-}$ mice are larger and hypercellular, over proliferative with hyperchromatic and pseudostratified nuclei and have significantly elevated number of neutrophils compared to $Rag1^{-/-}$ mice (76). As a result, $HCA_2^{-/-}$ $Rag1^{-/-}$ mice have significantly higher colitis scores for colons and cecum compared to $Rag1^{-/-}$ mice (76). Importantly, $HCA_2^{-/-}$ $Rag1^{-/-}$ mice have significantly increased numbers of ILC3 in the colonic lamina propria, mesenteric lymph nodes and small intestine, leading to a markedly higher frequency of IL-17 in the colonic lamina propria and mesenteric lymph nodes (76). Niacin significantly decreases IL-23 production by colonic DCs and the numbers of ILC3 in $Rag1^{-/-}$ mice, but fails to do so in $HCA_2^{-/-}$ $Rag1^{-/-}$ mice (76). Furthermore, $HCA_2^{-/-}$ $Rag1^{-/-}$ mice present signs of ongoing adenomatous transformation in the cecum and colonize a higher portion of IBD associated bacteria including *Bacteroidaceae*, *Porphyromonadaceae*, *Prevotellaceae*, *Streptococcaceae*, *Christensenellaceae*, *Mogibacteriaceae*, *Enterobacteriaceae,* and *Mycoplasmataceae*. Depletion of gut microbiota by antibiotics alleviates colonic inflammation by decreasing production of IL-23 and induction of ILC3 in $HCA_2^{-/-}$ $Rag1^{-/-}$ mice (76).

Further detailed studies on the role of HCA_2 in DSS-induced colitis treatment demonstrate that $HCA_2^{-/-}$ mice are highly susceptible to colitis development, with all experimental animals succumbing to death 10 days after DSS administration (3). In contrast, WT counterparts all survive through the entirety of the DSS treatment (34). Sodium butyrate markedly reduces inflammation and improves IECs barrier integrity by activating HCA_2 signaling and suppressing the AKT-NF-κB p65 signaling pathway in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, a model that resembles Crohn's disease (51, 52). In a similar study, a sodium butyrate-containing diet attenuates diarrhea symptoms and facilitates tight junction protein expression in the colon of piglets by acting on Akt signaling pathway in an HCA_2-dependent manner (103). Another source of butyrate, tributyrin, is a chemically stable structured lipid that could be administered orally (104). Tributyrin supplementation prevents mice from chronic and acute ethanol-induced gut injury by improving gut barrier function (occludin, ZO-1) and increasing the expression of HCA_2 in both ileum and proximal colon (105). In accordance with this, niacin administration attenuates iodoacetamide-induced colitis by a reduction in colon weight and colonic myeloperoxidase activity (a hallmark of colonic inflammation), and restores normal levels of colonic IL-10, tumor necrosis factor alpha (TNF-α), angiostatin and endostatin in a rat model (106). This beneficial effect of niacin is largely abolished by mepenzolate bromide, a HCA_2 receptor blocker, indicating niacin/ HCA_2 signaling ameliorates iodoacetamide-induced colitis (106). In addition to it oral pharmacologic activity, niacin is also a microbial-derived metabolite, produced by specific gut microbiota, including *Lactobacillus acidophilus*, *Bacteroides fragilis*, *Prevotella copri*, *Fusobacterium varium*, *Clostridium difficile*, *Bifidobacterium infantis,* and *Ruminococcus lactaris* (76, 107, 108). Niacin deficiency is associated with intestinal inflammation and diarrhea (76).

Overall, these reports provide compelling evidence that HCA_2 signaling modulates immune cells to inhibit production of several inflammatory cytokines, pathways, and enzymes, leading to the suppression of experimental models of colitis.

ROLE OF HCA_2 IN COLON CANCER

HCA_2 not only plays a critical role in the suppression of intestinal inflammation, but also has a significant effect on colonic cancer development and progression. Expression of HCA_2 is silenced in colon cancer cell lines, and in both mice and humans with colon cancer (24). The tumor-associated silencing of HCA_2 involves DNA methyltransferase 1 (DNMT1)-mediated DNA methylation (24) (**Figure 3A**). Reexpression of HCA_2 in cancer cell lines induces apoptosis by inhibiting B-cell lymphoma (Bcl)-2, B-cell lymphoma-extra-large (Bcl-xL), cyclin D1 and NF-κB activity and upregulating the death receptor pathway in a ligand-dependent manner (**Figure 3B**). Butyrate is also an inhibitor of histone deacetylases, but this HCA_2-mediated effort in colon cancer cells does not involve repressing histone deacetylation (24). Strikingly, Bardhan et al. discovered that IFN-γ reverses DNA methylation-mediated HCA_2 silencing without altering the methylation status of the HCA_2 promoter in colon carcinoma cells (109). Signal transducer and activator of transcription 1 (STAT1) is rapidly activated by IFN-γ and binds to the p300 promoter to activate p300 transcription. p300 is a histone acetyltransferase and a master transcriptional mediator in mammalian cells, resulting in a permissive chromatin conformation at the HCA_2 promoter to allow STAT1 to activate HCA_2 transcription despite DNA methylation (109) (**Figure 3B**).

Consistently, $HCA_2^{-/-}$ mice are more susceptible to the development of colon cancer (34, 46, 106, 110). In mouse models of inflammation-associated colon cancer caused by AOM and DSS, colons of $HCA_2^{-/-}$ mice shrink with highly increased myeloperoxidase activity, upregulated expression of colon cancer-promoting genes such as cyclin-D1, cyclin-B1, and cyclin-dependent kinase 1, decreased tight junction proteins expression and decreased expression of genes that inhibit colitis and colon carcinogenesis, such as transforming growth factor beta (*Tgfb)*1, *Tgfb2*, Solute Carrier Family 5 Member 8 (*Slc5a8*), MutS Homolog (*Msh*)2, and *Msh3* (34) (**Figure 3C**). In addition, $HCA_2^{-/-}$ mice exhibit a severe impairment of IL-10 and IL-18 production when compared to WT counterparts (34, 111) (**Figure 3C**). Histologically, crypt and epithelium structure damage, mucosa ulcerations and large amount of immune cell infiltration is observed in colons of AOM+DSS treated

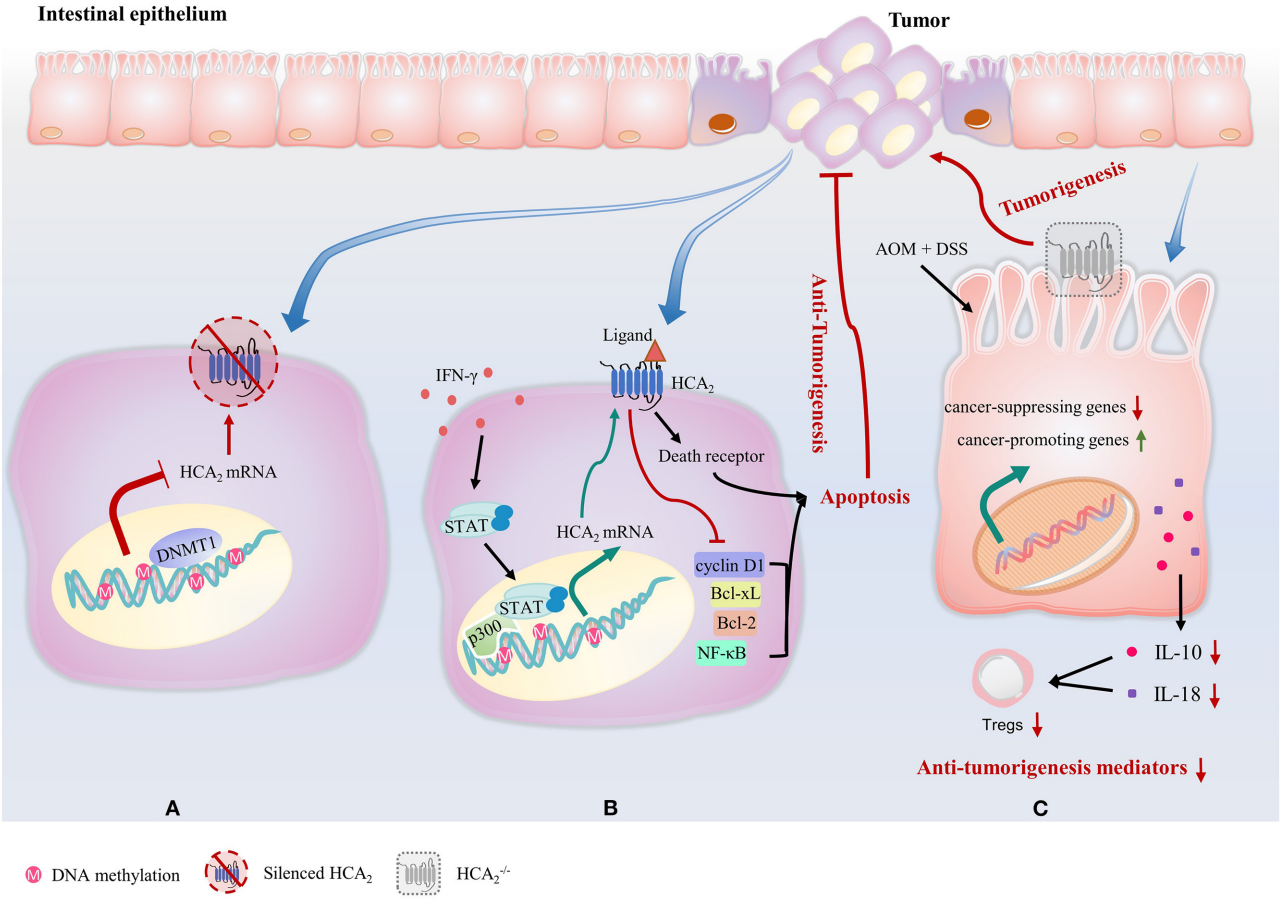

FIGURE 3 | HCA_2 plays a critical role in the suppression of colonic cancer. **(A)** Expression of HCA_2 is silenced in colon cancer cells. This tumor-associated silencing of HCA_2 involves DNMT1-mediated DNA methylation. **(B)** IFN-γ reverses DNA methylation-mediated HCA_2 silencing without altering the methylation status of the HCA_2 promoter. STAT1 is rapidly activated by IFN-γ and binds to the p300 promoter to activate p300 transcription, resulting in a permissive chromatin conformation at the HCA_2 promoter to allow HCA_2 transcription in colon carcinoma cells. Reexpression of HCA_2 in cancer cell lines induces apoptosis by inhibiting of Bcl-2, Bcl-xL, cyclin D1, and NF-κB activity and upregulating the death receptor pathway in a ligand-dependent manner. **(C)** In mouse models of inflammation-associated colon cancer caused by AOM and DSS, the intestinal epithelium of $HCA_2^{-/-}$ mice display upregulated expression of colon cancer-promoting genes and decreased genes that inhibit colitis and colon carcinogenesis. $HCA_2^{-/-}$ mice also exhibit a severe impairment of IL-10 and IL-18 production, which leads to a decrease in Treg cell number.

$HCA_2^{-/-}$ mice group, indicating epithelial barrier breakdown (34). Systemically, levels of both colonic and serum cytokines that promote colonic inflammation and carcinogenesis such as amyloid A, chemokine (C-X-C motif) ligand (CXCL) 1, C-C motif chemokine ligand (CCL) 2, IL-1β, IL-6, and IL-17 are all elevated. At the end of the AOM+DSS treatment regime, $HCA_2^{-/-}$ mice demonstrate anemia and increased number of large polyps on colon (34). Remarkably, niacin administration suppresses colon tumor development in antibiotic-treated microbiota-depleted WT mice (34). However, it also promotes colitis-associated cancer in $HCA_2^{-/-}$ mice, which is associated with an expansion of bacteria in *Prevotellaceae* family and TM7 phylum (34), suggesting microbiota/niacin protective effect is HCA_2-dependent. In the same report, Singh et al. manipulated another mouse model of intestinal carcinogenesis, $Apc^{Min/+}$, in which multiple intestinal neoplasia (*Min*) is a mutant allele of the murine adenomatous polyposis coli (*Apc*) locus (110). $Apc^{Min/+}$ mice show significantly enlarged colonic polyp numbers, which were rescued by niacin treatment. However, niacin was not able to decrease the development of colonic polyps in $HCA_2^{-/-}Apc^{Min/+}$ (34).

Taken together, these data demonstrate that HCA_2 mediates cancer development and progression by promoting intestine mucosal immunity and decreasing cancer-promoting genes.

ANTI-INFLAMMATORY EFFECTS OF HCA_2 IN OTHER DISEASES

HCA_2 signaling plays an essential role in preventing and reducing inflammation in the intestine. In addition, HCA_2 has also been associated with anti-inflammatory effects in numerous disease states. In particular, various studies report that activation of HCA_2 reduces inflammation in atherosclerosis (36), diabetes mellitus (25), diabetic retinopathy (31), neurodegenerative diseases (37, 38), sepsis (39), mammary cancer (40) and

pancreatitis (41). Activation of HCA_2 on immune cells in the vasculature by niacin reduces the progression of atherosclerosis and suppresses macrophage recruitment to atherosclerotic plaques (36). Chronic activation of HCA_2 by niacin increases serum adiponectin in obese men with metabolic syndrome, suggesting a role in diabetes mellitus and obesity (112, 113). Additionally, in pancreatic islets of diabetic db/db mice as well as in type 2 diabetic (T2D) patients, HCA_2 expression is decreased (114). Administration of GSK256073, a HCA_2 agonist, notably reduced serum glucose and non-esterified fatty acids without inducing the niacin-associated side effect of cutaneous flushing in diabetic patients (25). In retinal pigmented epithelial cells, niacin-mediated activation of HCA_2 suppresses TNF-α-induced NF-κB activation and IL-6 and monocyte chemoattractant protein-1 (MCP-1) secretion (31). HCA_2 ligands have been also reported to attenuate inflammation in neurodegenerative diseases such as Parkinson's disease (115), Huntington's disease (38), Alzheimer's disease (116), multiple sclerosis (37), ischemic stroke (117) and traumatic brain injury (118), although, the mechanisms behind many of these beneficial effects have yet to be fully elucidated. In sepsis, niacin attenuated kidney and lung inflammation by decreasing NF-κB activation and subsequently decreasing inflammatory cytokines (39, 119, 120). As was the case in colon cancer, HCA_2 functions as a tumor suppressor in mammary cancer via inhibition of genes involved in cell survival and anti-apoptotic pathways in human breast cancer cell lines (40). In pancreatitis, β-OHB supplementation inhibits macrophage NF-κB activation in an HCA_2-dependent manner, and limits sterile inflammation (41). Moreover, HCA_2 plays an antiviral role in reducing the Zika virus replication. HCA_2 expression is significantly induced by Zika virus infection, while depletion of HCA_2 resulted in significant increase of Zika virus RNA levels and viral yields, indicating that HCA_2 can serve as a restriction factor for Zika virus and providing a potential target for anti- Zika virus therapeutic (121).

CONCLUSION

There is mounting evidence summarized in this review that HCA_2 plays an important role in modulating inflammation and carcinogenesis in the intestine. Ligands for the HCA_2 receptor mediate a wide variety of inflammation-suppressing signaling events. NF-κB, NLRP3 and prostaglandins PGD_2 and PGE_2 have all been implicated as downstream targets of the HCA_2 receptor, suggesting activation of one pathway may have beneficial or undesirable effects that are tissue-dependent. Therefore, tissue-specific, pharmacologic ligands which trigger bias signaling cascades, and therefore minimize less desirable downstream effects, are required. In addition, these pathways interweave the process of inflammatory and metabolic disorders through HCA_2. Thus, this interplay of gut microbiota, HCA_2 signaling and immune responses is a double-edged sword of inducing inflamed intestinal diseases or colon cancer and promoting intestinal homeostasis.

AUTHOR CONTRIBUTIONS

ZL was primarily responsible for researching and writing the manuscript (including the generation of figures). KM was responsible for writing specific sections and reviewing the manuscript. RJ proposed the topic of the review and supervised the writing and review of the manuscript. All authors contributed to the article and approved the submitted version.

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