Symbiotic polyamine metabolism regulates epithelial proliferation and macrophage differentiation in the colon

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Intestinal microbiota-derived metabolites have biological importance for the host. Polyamines, such as putrescine and spermidine, are produced by the intestinal microbiota and regulate multiple biological processes. Increased colonic luminal polyamines promote longevity in mice. However, no direct evidence has shown that microbial polyamines are incorporated into host cells to regulate cellular responses. Here, we show that microbial polyamines reinforce colonic epithelial proliferation and regulate macrophage differentiation. Colonisation by wild-type, but not polyamine biosynthesis-deficient, Escherichia coli in germ-free mice raises intracellular polyamine levels in colonocytes, accelerating epithelial renewal. Commensal bacterium-derived putrescine increases the abundance of anti-inflammatory macrophages in the colon. The bacterial polyamines ameliorate symptoms of dextran sulfate sodium-induced colitis in mice. These effects mainly result from enhanced hypusination of eukaryotic initiation translation factor. We conclude that bacterial putrescine functions as a substrate for symbiotic metabolism and is further absorbed and metabolised by the host, thus helping maintain mucosal homoeostasis in the intestine.

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Polyamines, which are aliphatic compounds containing more than two amino groups, are widely distributed among prokaryotes and eukaryotes, including humans, and regulate multiple biological processes, including translation, transcription and cell proliferation and differentiation. Among polyamines, putrescine and spermidine are especially prevalent in the large intestine of human and mice. We previously reported polyamines, putrescine and spermidine are especially prevalent in mice by inducing autophagy and improves cardiac dysfunction as well as metabolic syndrome in mice. Likewise, exogenous spermidine serves as a substrate for hypusine, in which the aminobutyl group is decarboxylated (S-adenosyl methionine). Spermidine is generated from putrescine by addition of aminopropyl groups derived from decarboxylated S-adenosyl methionine. Spermidine serves as a substrate for hypusine, in which the aminobutyl group of spermidine is attached to a specific lysine residue. Hypusination of eukaryotic translation initiation factor 5 A (eIF5A) is required for its activation and hypusinated eIF5A (hyp-eIF5A) is involved in translation initiation, elongation, termination, and cell cycles.

The intestinal epithelium is one of the most rapidly self-renewing tissues in adult mammals, with cell turnover every 3–5 days. Microbial colonisation induces rapid epithelial turnover in the intestine. Because intestinal epithelial cells are constantly exposed to pathogens and harmful components from diets or microbiota, this rapid turnover promotes elimination of infected and/or damaged cells. Previous studies demonstrated the importance of polyamine for proliferation and wound healing in the intestinal epithelium, whereas contribution of luminal polyamines to epithelial cell turnover has not been directly explored.

Polyamines also regulate immune responses. For example, spermidine restores CD8+ T cell responses in elderly mice. Further, the treatment with spermine inhibits lipopolysaccharide (LPS)-mediated production of nitric oxide and pro-inflammatory cytokines, such as tumour necrosis factor (TNF), interleukin (IL)-1β, and IL-6 in mouse macrophages and human mononuclear cells. Recent studies have demonstrated that deleting ODC enhances classical activation of M1 macrophages. Conversely, polyamine synthesis is enhanced in alternatively activated M2 macrophages by IL-4 stimulation. Compared with other organs, larger numbers of macrophages reside in the intestines, accumulating in the colonic lamina propria (cLP) near the epithelial layer. Under physiological conditions, there are two major macrophage/microbe subsets, inflammatory CCR1lowLy6C+ and anti-inflammatory CCR1highLy6C− cells in the cLP. While inflammatory CCR1lowLy6C+ cells are involved in biological defence in the intestinal mucosal barrier, anti-inflammatory CCR1highLy6C− cells play roles in immune tolerance to suppress excessive immune responses. However, it remains unknown whether polyamines influence the development of these two myeloid populations in the colon.

In this study, we evaluated the biological significance of commensal bacterium-derived polyamines. We established a gnotobiotic mouse associated with the putrescine synthesis genes, which lacks genes for putrescine synthesis (Fig. 1b). Potrcine was nearly absent in the SK930 culture supernatant, and spermidine concentrations in the wild-type (WT) and SK930 culture supernatants were comparable to that in the Luria-Bertani (LB) medium (Supplementary Fig. 2a) with no defects in growth or LPS synthesis (Supplementary Fig. 2b, c). We inoculated the WT and SK930 strains into GF mice (F0), then generated F1 gnotobiotic mice with and without bacterium-derived polyamines. We obtained gnotobiotic mice with and without bacterium-derived polyamines may be incorporated into colonocytes and may partly contribute to hyperproliferative responses. We evaluated this hypothesis using a gnotobiotic mouse system with putrescine synthesis-deficient or -sufficient bacteria. Certain commensal bacteria, such as E. coli, produce putrescine via two pathways using arginine and ornithine as substrates (Fig. 1b). Although E. coli is a minor member of the intestinal microbiota in adult humans, this species is prominently enriched in the microbial community early in life when the intestine is rapidly maturing.

To assess the contribution of bacterial putrescine to epithelial proliferation, we used E. coli SK930, which lacks genes for putrescine synthesis (Fig. 1b). Potrcine was nearly absent in the SK930 culture supernatant, and spermidine concentrations in the wild-type (WT) and SK930 culture supernatants were comparable to that in the Luria-Bertani (LB) medium (Supplementary Fig. 2a) with no defects in growth or LPS synthesis (Supplementary Fig. 2b, c). We inoculated the WT and SK930 strains into GF mice (F0), then generated F1 gnotobiotic mice via maternal-infant transmission to determine the effect of bacterial putrescine on the intestinal physiology (Fig. 1c).

We obtained gnotobiotic mice with and without bacterium-derived putrescine (Fig. 1d), in which the number of E. coli in the luminal contents were comparable throughout the jejunum to the distal colonic lumen in both mouse groups, as were the levels of other metabolites, including short-chain fatty acids (SCFAs; Supplementary Fig. 3a–c and Supplementary Table 1). Notably, WT-strain-associated, but not SK930-strain-associated, mice exhibited significantly increased intracellular levels of putrescine and its derivative, spermidine, in CECs (Fig. 1e).
implying that bacterial putrescine is metabolised to spermidine in CECs. Moreover, CEC proliferation was significantly enhanced in WT-strain-associated mice compared with that in the GF and SK930-strain-associated mice (Fig. 1f and Supplementary Fig. 3d). SK930 colonisation also slightly increased the CEC proliferation likely due to the stimulation from LPS and/or lactate (Supplementary Table 1), both of which have been reported to accelerate CEC proliferation43,44.

**Fig. 1 Bacterial putrescine facilitates epithelial cell proliferation in the colon.** a Polyamine concentrations in faeces of eight-weeks old GF and SPF mice (n = 5). b Polyamine synthesis pathway in *E. coli* and the deleted genes (marked with X) in an *E. coli* strain SK930. Blue letters represent enzymes and transporters: arginine decarboxylase (SpeA), agmatine ureohydrolase (SpeB), ornithine decarboxylase (SpeC and SpeF), adenosylmethionine decarboxylase (SpeD), spermidine synthase (SpeE), putrescine-ornithine antiport transporter (PotE) and putrescine exporters (SapBCDF). c Experimental design for gnotobiotic mice colonised with WT or SK930 strain. d The polyamine concentrations in faeces of F1 mice. e The polyamine concentrations in CECs (n = 8, independent animals). f Representative microscopic images of EdU assay and quantification of EdU-positive cells/crypt in each group. EdU-positive cells (green). Nuclei are counterstained with DAPI (blue) (n = 8, independent animals). Scale bars: 100 µm. Three randomly selected areas were examined per slide (n = 13). All data shown represent the mean ± SEM. Statistical significance was calculated using a the Welch’s t-test (two-sided), d, e Kruskal–Wallis followed by Steel–Dwass post hoc test, f one-way ANOVA followed by Tukey’s post-hoc test.

**CECs actively utilise luminal putrescine.** To confirm whether dietary putrescine reaches the colon, mice were orally administered stable-isotope-labelled \(^{15}\)N\(_2\)-putrescine in phosphate-buffered saline (PBS) containing blue food colouring agent as a marker to determine the position of the labelled-putrescine in the digestive tract. We analysed the isotope ratio of polyamines in the blue-stained luminal contents on arrival in the ileum (2 h after oral administration) and in the colon (3.5 h after oral administration).
administration; Supplementary Fig. 4a, b). The labelled-putrescine/total-putrescine ratio was 35.0% in the ideal luminal contents 2 h after oral administration. Conversely, only minimal labelled putrescine (0.36%) was detected in the colonic luminal contents after 3.5 h (Supplementary Fig. 4c). These results demonstrated that a large portion of dietary putrescine was absorbed in the upper digestive tract before reaching the colonic lumen. To assess the uptake of luminal putrescine and its conversion to spermidine in CECs, we injected labelled putrescine into the colonic lumens of mice (luminal administration model; Supplementary Fig. 4d). Two hours post-injection, we compared the isotope ratios of the polyamines in the CECs with those in the CECs collected 2 and 3.5 h after oral administration, when the putrescine solution reached the ileum and colon, respectively (oral-administration model; Supplementary Fig. 4a, c). Isotope analysis revealed that the labelled-putrescine/total-putrescine ratio was 11.2% in the CECs in the luminal-administration model (Supplementary Fig. 4e). Conversely, the labelled-putrescine/total-putrescine ratios were only 2.96% and 2.23% in the CECs 2 and 3.5 h after oral administration, respectively. Additionally, labelled spermidine was higher in the luminal-administration-model CECs than in the oral-administration-model CECs (Supplementary Fig. 4e). However, 2 h after oral administration, when the putrescine had reached the ileum but not the colon, low levels of labelled putrescine were detected in the CECs, indicating that the oral putrescine may have reached the CECs through the bloodstream. Only a portion of the dietary putrescine was transported to the CECs, suggesting that exogenous local putrescine in the colonic lumen is actively incorporated and used as a polyamine-metabolic source for CECs.

Labelled putrescine was detected in the portal vein of the luminal-administration model (Supplementary Fig. 4f). Thus, intestinal bacterium-derived putrescine may affect the whole body. To further confirm that CECs use exogenous polyamines, we incubated intestinal organoids generated from colonic crypts in a standard medium supplemented with 100 μM 15N2-putrescine for 2 days. The intracellular isotope-labelled polyamines, 15N2-putrescine and 15N2-spermidine, occupied 47 and 30%, respectively, of the corresponding polyamines in the organoids (Fig. 2a, b). Thus, exogenous putrescine was actively uptaken and converted to spermidine in the CECs. Importantly, we detected putrescine at 132.3 ± 17.3 μM in the standard medium; this concentration was similar to that of the luminal putrescine (Fig. 1a, d). Notably, the commercial organoid culture medium and supplements (Dulbecco’s modified Eagle’s medium [DMEM]/F12 supplemented with B27 and N2) also contained similar levels of putrescine. Thus, intestinal organoid growth may require exogenous putrescine. Treatment with trans-4-methylcyclohexylamine (MCHA; Fig. 2c), a spermidine synthase (SPDS) inhibitor, diminished the organoid growth (Fig. 2d). Thus, epithelial proliferation likely requires exogenous putrescine as a substrate for spermidine synthesis.

Hypsination of eIF5A mediates the polyamine-dependent epithelial proliferation. We subsequently explored the underlying mechanism by which polyamines regulate host epithelial proliferation. Ki67-positive proliferative CECs were increased in WT-strain-associated mice (Supplementary Fig. 3d). Nevertheless, mRNA expressions of Mki67 (encoding Ki67) did not significantly differ between the WT- and SK930-strain-associated mice (Fig. 2e). Thus, polyamines may augment proliferation-related molecules via post-transcriptional regulation. Hyp-eIF5A, for which spermidine serves as a substrate, is involved in post-transcriptional regulation, including translation initiation, elongation, and termination. Therefore, we thought that MCHA-mediated inhibition of organoid growth may result from the reduction of hyp-eIF5A levels. Indeed, MCHA treatment decreased the hyp-eIF5A levels in the organoids, and exogenous spermidine rescued this decrease (Fig. 2f), indicating that the reduced hyp-eIF5A due to the inhibited spermidine synthesis arrested organoid growth. However, because the organoid growth was significantly suppressed, but hyp-eIF5A was only moderately reduced, MCHA may inhibit growth via other mechanisms in addition to reducing hyp-eIF5A.

We conducted organoid experiments to evaluate the difference in hyp-eIF5A levels in the CECs of two gnotobiotic mouse models. Colonisation by the WT strain, but not the SK930 strain, significantly increased the hyp-eIF5A levels in the CECs (Fig. 2g). To verify the contribution of hyp-eIF5A to epithelial proliferation, we treated colonic organoids with N1-monoguanyl 1,7-diaminoheptane (GC7), which inhibits deoxyhypusine synthase (DHS) necessary for hyp-eIF5A synthesis (Fig. 2c). This treatment dose-dependently suppressed the organoid growth in conjunction with reducing the hyp-eIF5A levels (Fig. 2h, i), indicating that hyp-eIF5A deficiency suppressed organoid growth. As reported by Lou et al., GC7 exhibits little cytotoxicity up to 20 μM, whereas higher GC7 concentrations (50 – 100 μM) significantly inhibit hepatocellular carcinoma cell viability. Therefore, we believe that the growth arrest of colonic organoids by 15 μM of GC7 did not result from the cytotoxicity of GC7. Furthermore, orally administering GC7 to SPF mice via their drinking water for 5 days markedly reduced the hyp-eIF5A levels and EdU-positivity in the CECs compared with those of the control mice who received regular tap water (Fig. 2j, k). Thus, GC7 suppressed CEC proliferation with decreased hyp-eIF5A levels both in vivo and in vitro.

Autophagy is closely related to hyp-eIF5A and is associated with proliferation and differentiation. Autophagy is also activated in the crypts, which are proliferating regions, in CECs. To assess whether putrescine produced by the intestinal microbiota increases autophagy activity, we detected LC3 and SQSTM1/p62 (p62) in CECs located 50 µm from the bottom of the crypt via immunohistochemical analysis. WT-strain-associated mice exhibited more LC3 dots than did the SK930-strain-associated mice (Fig. 3a). Additionally, the WT-strain-associated mice exhibited significantly more p62 dots and LC3-p62 double-positive dots than did the SK930-strain-associated mice (Fig. 3a, b). The LC3, p62, and LC3-p62 dots represent autophagosomal formation, sequestosomal formation (which is selectively degraded by autophagy), and autophagosome/autolysosome formation (incorporation of p62-positive sequestosomes into the autophagosome), respectively. Thus, autophagy was activated in the CECs of WT-strain-associated mice compared with that in the CECs of SK930-strain-associated mice.

Hyp-eIF5A promotes efficient expression of mitochondrial oxidative phosphorylation (OXPHOS) proteins. Therefore, we evaluated OXPHOS protein complexes I–V in the CECs of gnotobiotic mice. CI was significantly higher, and CII, CIV, and CV tended to be higher in SK930-strain-associated mice than in the WT-strain-associated mice, indicating that enhanced hyp-eIF5A did not promote OXPHOS in the WT-strain-associated mice (Fig. 3c). Crypt cells depend on glycolysis more than on OXPHOS for ATP production, whereas the dependence of enterocytes on OXPHOS to fulfil their energetic requirements increases with differentiation and maturation. Therefore, these results were likely obtained because the WT-strain-associated mice exhibited more proliferating cells in the CEC crypts via polyamines derived from the WT strain than did the SK930-strain-associated mice. Thus, in this case, cell proliferation was likely more strongly influenced by the difference in energy metabolism in the CECs than by the hyp-eIF5A–OXPHOS axis.
Bacterium-derived polyamines regulate macrophage balance in the colon. A previous study found that polyamines downregulate pro-inflammatory molecules, including nitric oxide (NO) and TNF, in LPS-stimulated macrophages and microglia. In support of this view, ablation of ODC, a rate-limiting enzyme in polyamine synthesis, augments pro-inflammatory cytokines and chemokines in macrophages, reinforcing the M1 macrophage phenotype. M2 macrophages, which play vital roles in tissue remodelling and resolution of inflammatory responses, are characterised by expression of arginase-1 (Arg1), which produces...
ornithine. Therefore, we hypothesised that commensal-derived polyamines might significantly affect the M1/M2 macrophage balance. Under physiological conditions, two major macrophage/monocyte populations exist in the colonic lamina propria (cLP): CX3CR1lowLy6C+ cells and CX3CR1highLy6C− cells, which display inflammatory and anti-inflammatory properties, respectively. WT strain colonisation was increased in the CX3CR1highLy6C− subset in the cLP but not in the spleen (Fig. 4a–c and Supplementary Fig. 5a, b). Additionally, an Arg1+M2-like subset was significantly increased in the WT-strain-associated mice compared with that in the GF mice. Conversely, association with the SK930 strain failed to affect the CX3CR1highLy6C− subset and significantly increased the frequency of NOS2+M1-like macrophages in SK930-strain-associated mice, thus augmenting the M1/M2 ratio in the cLP (Fig. 4d–g).

Bacterial putrescine exerts a localized effect in regulating the macrophage balance. To determine whether exogenous polyamines directly regulate macrophage polarisation, bone marrow-derived macrophages (BMDMs) were cultured in medium with or without putrescine. Similar to the results of the WT- and SK930-strain-associated mice, exogenous putrescine upregulated CX3CR1 expression and downregulated Ly6C expression in the BMDMs (Fig. 4h, i). To further confirm that macrophages take up exogenous polyamines, we incubated BMDMs in a complete RPMI medium supplemented with 100 μM 15N2-putrescine for 24 h. The intracellular isotope-labelled polyamines, 15N2-putrescine and 15N2-spermidine, occupied 81.5% and 60.6%, respectively, of the corresponding polyamines in the BMDMs (Fig. 4j, k), demonstrating that exogenous putrescine was actively uptaken and converted to spermidine in the BMDMs.

A recent study revealed that polyamine biosynthesis causes eIF5A hypusination in macrophages, and hyp-eIF5A modulates expression of OXPHOS-related mitochondrial proteins to induce alternative macrophage activation. To assess the effect of exogenous putrescine on hyp-eIF5A and OXPHOS, we treated bone marrow cells with macrophage colony-stimulating factor and the presence or absence of DFMO and putrescine. Hypusination of eIF5A was upregulated in macrophages stimulated with IL-4, but not in those stimulated with LPS + IFN-γ, which is similar to the results of previous studies (Supplementary Fig. 6). DFMO treatment decreased hyp-eIF5A in BMDMs, and exogenous putrescine rescued this DFMO-induced reduction in hyp-eIF5A. Meanwhile, exogenous putrescine treatment without DFMO slightly increased the hyp-eIF5A levels. We further analysed whether polyamines cause metabolic reprogramming in BMDMs. The expressions of OXPHOS complex proteins CI−CV were assessed via western blotting. CI, CII and CIV were upregulated in macrophages stimulated with IL-4, but not in those stimulated with LPS + IFN-γ, consistent with the previous studies (Supplementary Fig. 6). Additionally, CI, CII and CIV expressions were decreased in DFMO-treated macrophages, and exogenous putrescine restored this suppression. OXPHOS complex protein expression in macrophages treated only with putrescine resembled that in macrophages stimulated with IL-4 (Supplementary Fig. 6). Thus, exogenous putrescine induced metabolic reprogramming similar to that of IL-4 stimulation.

**Bacterium-derived polyamines mitigate DSS-induced colitis.** Vigorous epithelial proliferation is a fundamental mucosal barrier function. CX3CR1highLy6C− macrophages contribute to suppressing inflammatory responses in the intestine. Therefore, we explored whether bacterial polyamines may substantially contribute to maintaining intestinal immune homeostasis. We administered DSS in the drinking water to F1 gnotobiotic mice to induce experimental colitis. The number of *E. coli* during DSS-induced colitis did not differ between the WT- and SK930-associated mice (Supplementary Fig. 7a); however, WT-strain-associated mice were more resistant to the DSS-induced colitis than were the SK930-strain-associated mice, as evidenced by the significant amelioration of disease symptoms and increased survival rates in the WT-strain-associated mice (Fig. 5a–c). The WT-strain-associated mice also exhibited alleviated colonic thickening, histological scores and faecal lipocalin-2 levels (Fig. 5d–f). Flow cytometry showed an accumulation of CD11b+ICAM4+80+ macrophages/monocytes (Supplementary Fig. 7b, c), especially the CX3CR1lowLy6C+ and NOS2+ M1-like subsets, in the cLPs of the SK930-strain-associated mice (Fig. 5g–i and Supplementary Fig. 7d–f). Conversely, colonisation by the WT strain decreased the M1/M2 macrophage balance under inflammatory conditions (Fig. 5j). Thus, bacteria-derived polyamines play anti-inflammatory roles in the intestinal immune system. Interestingly, a recent study demonstrated that spermine oxidase deficiency reduced colonic spermidine levels, which exacerbated lethality and mucosal inflammation, in a DSS-induced colitis model. This observation supports the importance of polyamine metabolism in maintaining intestinal immune homeostasis. Of note, a recent work exhibited that gastrointestinal pathobionts such as *Helicobacter pylori* and *Citrobacter rodentium* induce accumulation of the intracellular hyp-eIF5A level in macrophages by upregulating DHS. DHS-deficient macrophages are defective in antibacterial response to these pathobionts, suggesting that polyamine-dependent formation of hyp-eIF5A is essential to prevent bacterial infection on the gastrointestinal mucosa.
Carriche et al. revealed that spermidine promoted differentiation into CD4+Foxp3+ T cells in vitro, and oral administration of spermidine increased CD4+Foxp3+ T cells in the small intestines and cLP in vivo. However, the CD4+Foxp3+ T cells did not significantly differ between the cLPs of the GF, WT- and SK930-strain-associated mice in our experiments (Supplementary Fig. 5c). Therefore, CD4+Foxp3+ cells were not involved in the results of the DSS-induced colitis experiment in this study.

To our knowledge, this study provides the first direct evidence that host cells incorporate polyamines from the colonic lumen, which eventually increase intracellular polyamine levels to regulate the mucosal epithelium and immunity. Recent studies exhibited that ODC expression was downregulated in the inflamed region compared with that in the unoinflamed region of the colonic mucosa in patients with Crohn’s disease. Thus, decreased endogenous polyamine synthesis might be involved in the development and/or exacerbation of inflammatory bowel disease (IBD). Therefore, our study may suggest an alternative therapeutic strategy for IBD and metabolic syndrome by manipulating microbial polyamine synthesis using prebiotics, probiotics, or synbiotics (e.g., arginine and Biﬁdobacterium spp.)30. Notably, polyamine biosynthesis is highly upregulated in cancerous cells, and selective inhibition of ODC is effective in treating colon cancer. This suggests the pathological relevance

**Fig. 3** Bacterial putrescine activates autophagy and OXPHOS in CECs. a, b Representative microscopic images of LC3 and p62 and its enlarged view. Green and red dots indicate LC3 and p62, respectively. Nuclei are stained with Hoechst (blue). The white arrowhead indicates LC3-p62 double-positive dots. Scale bars: 10 µm. Three randomly selected areas were examined per slide (WT: n = 8, SK930: n = 6, independent animals). c Western blot analysis of OXPHOS complex I-V (CI-CV) in CECs of gnotobiotic mice (n = 3, independent animals). All data shown represent the mean ± SEM. Statistical significance was calculated using the Welch’s t-test (a, p62 and c) (two-sided), Mann–Whitney test with (a, LC3 and b, LC3-p62) (two-sided).
of polyamines produced by commensals in colorectal carcinogenesis, although further investigation is required to confirm this notion.

In conclusion, low-molecular-weight metabolites produced by the intestinal microbiome are absorbed from the colonic lumen and carried into the systemic circulation. Some of these metabolites play vital roles in the health and diseases of host animals. Accumulating evidence shows that bioactive metabolites, such as SCFAs, act directly on host cells to exert physiological functions. Additionally, host cells may further metabolise certain
Fig. 4 Bacterial putrescine regulates the development of macrophage subsets. CX3CR1 and Ly6C expression were analysed in CD45+CD11b+F4/80+ cells via flow cytometry. Representative flow cytometry images (a), frequency (b), number (c) of CX3CR1lowLy6C+ monocyte/macrophage and CX3CR1highLy6C− macrophages and representative flow cytometry images (d), frequency (e), number (f) of NOS2+Arg1− and NOS2−Arg1+ macrophages and the ratio of the former to the latter subset (g) in cLP of gnotobiotic mice colonised with WT (n = 7) or SK930 (n = 8) strain and GF mice (n = 6). CX3CR1 and Ly6C expression in CD45+CD11c−CD11b+F4/80+ cells from BMDMs cultured in medium with or without putrescine (n = 4). Representative flow cytometry images (h) and frequency (i, j). Isotope ratio of putrescine and spermidine in BMDMs cultured with isotope-labelled15N2-putrescine were analysed via GC-MS. The black bar indicates the percent putrescine (PUT) or spermidine (SPD) of15N2-putrescine or15N2-spermidine enrichment in total corresponding polyamines, respectively (n = 3, independent cultures). All data shown represent the mean ± SEM. Kruskal-Wallis followed by Steel-Dwass post hoc test (b–g) and Student’s t-test (i) (two-sided).

Fig. 5 Bacterial putrescine ameliorates DSS-induced colitis. 2% DSS in the drinking water to the F1 gnotobiotic mice colonised WT or SK930 strain to induce experimental colitis for six days, then regular drinking water thereafter. a–c: Red and blue indicate WT-strain-associated and SK930-strain-associated mice. a DAI score. b Body weight changes were measured daily and calculated as the percentage change from day 0. c Survival rate in mice after DSS administration. The survival curve was calculated using the Kaplan-Meier method and statistical significance was calculated using the log rank test (WT: n = 10, SK930: n = 8). d–j Mice were analysed on day 5 after starting 2% DSS (n = 10). d Representative image of the colons and values of weight/length of the colon. e Haematoxylin and eosin-stained images of representative histopathologic changes in the WT and SK930 strain-associated mice. Scale bar: 100 µm. Mucosal damage was estimated using a histological scoring system. f Faecal lipocalin-2 concentrations in faeces at day 5 after starting 2% DSS. Expression of CX3CR1 and Ly6C were analysed in CD45+CD11b+F4/80+ cells via flow cytometry. Representative flow cytometry images (g), and frequency (h) and number (i) of CX3CR1lowLy6C+ monocyte/macrophage and CX3CR1highLy6C− macrophages and the ratio of the former to the latter subset (j) in the cLP of gnotobiotic mice colonised with WT or SK930 strain (WT: n = 8, SK930: n = 9). All data shown represent the mean ± SEM. Statistical significance was calculated using the Welch’s t-test (e, h, i) (two-sided) with Bonferroni adjustments (a), Mann-Whitney test with Bonferroni adjustments (b), Student’s t-test (d, f, j) (two-sided) (*P < 0.05, **P < 0.01, ***P < 0.001).
symbiotic metabolism. Thus, our study revealed that symbiotic metabolism composed of both microbes and host cells could be termed symbiotic metabolism. Meanwhile, a part of inactive metabolites may be further converted to bioactive substances via host metabolic pathways after absorption (Metabolite C). The entities of such symbiotic metabolism, however, remain obscure. Our study using gnotobiotic mice demonstrated that microbial putrescine is uptaken by the colonic epithelial cells to produce bioactive spermidine that accelerates epithelial renewal and increases the abundance of anti-inflammatory macrophages in the colon. Thus, microbial putrescine serves as a source (precursor) of bioactive substances in the host animals. This observation provides evidence for symbiotic metabolism contributing to the maintenance of intestinal homeostasis.

**Methods**

**Bacterial strains.** *Escherichia coli* MG1655 was used as a wild-type (WT) strain. A triple mutant SK930 harbouring the deletion of putrescine synthesis genes encoding agmatine ureohydrolase (*speF*) and ornithine decarboxylase (*speC* and *speB*), was used as the knockout strain.

**Animal experiments.** All mice were purchased from Clea Japan, Inc. GF (IQR/Iec) mice were purchased and bred at Kyodo Milk Industry Co., Ltd. Faecal microbiome transplantation experiment: Mice were housed in plastic cages under a 12-hour light/dark cycle at 25 ± 2 °C with 50% ± 10% humidity. Mice were provided with sterilised water and sterilised low-polyamine pellet chow (Clea Japan, Inc.). To perform faecal microbiome transplantation, eleven-week-old GF male mice were inoculated in the stomach via a catheter with a suspension of the faeces obtained from the SPF mice. The exGF and SPF mice were then analysed for metabolic analysis of the caecal contents and CECs at 3, 6, 9, and 23 days after inoculation. Gnotobiotic mouse experiment: SPF mice were then analysed for metabolomic analysis of the caecal contents and CECs at 3, 6, 9, and 23 days after inoculation. Gnotobiotic mouse experiment: SPF mice were then analysed for metabolomic analysis of the caecal contents and CECs at 3, 6, 9, and 23 days after inoculation. Gnotobiotic mouse experiment: SPF mice were then analysed for metabolomic analysis of the caecal contents and CECs at 3, 6, 9, and 23 days after inoculation. Gnotobiotic mouse experiment: SPF mice were then analysed for metabolomic analysis of the caecal contents and CECs at 3, 6, 9, and 23 days after inoculation.

**Measurement of polyamine concentrations.** To determine the polyamine concentrations, each of the strains were grown under anaerobic conditions at 37 °C for 24 h in LB medium (BD Biosciences, USA) containing 2 mML-arginine, 1.5 g L−1 D-glucose, and 0.5 g L−1 L-cysteine-hydrochloride in screw-top test tubes with butyl rubber inner plugs. The gas-phase portions in screw-top test tubes were replaced with N2/CO2 (80:20, v/v) before autoclaving (115 °C, 15 min). E. coli precultures were harvested after 24 h and inoculated at a final OD600 of 1.0 × 10−2. To determine the growth curve and the amount of LPS, each strain was grown under aerobic conditions at 37 °C in LB medium. The growth curve was measured for up to 24 h using an absorption spectrophotometer with OD600. LPS was extracted when OD600 reached 0.3 by hot phenol-water method, and quantified using ToxinSensor Chromogenic LAL Endotoxin Assay Kit (GenScript Inc., USA) following the manufacturer’s protocol.

**Cultures of bacteria.** To determine the polyamine concentrations, each of the strains were grown under anaerobic conditions at 37 °C for 24 h in LB medium (BD Biosciences, USA) containing 2 mML-arginine, 1.5 g L−1 D-glucose, and 0.5 g L−1 L-cysteine-hydrochloride in screw-top test tubes with butyl rubber inner plugs. The gas-phase portions in screw-top test tubes were replaced with N2/CO2 (80:20, v/v) before autoclaving (115 °C, 15 min). E. coli precultures were harvested after 24 h and inoculated at a final OD600 of 1.0 × 10−2. To determine the growth curve and the amount of LPS, each strain was grown under aerobic conditions at 37 °C in LB medium. The growth curve was measured for up to 24 h using an absorption spectrophotometer with OD600. LPS was extracted when OD600 reached 0.3 by hot phenol-water method, and quantified using ToxinSensor Chromogenic LAL Endotoxin Assay Kit (GenScript Inc., USA) following the manufacturer’s protocol.

**Measurement of polyamine concentrations.** To prepare the polyamine extract, faeces were collected and stored at −80 °C until use. Frozen faeces (approximately 50 mg) were diluted 10-fold with Dulbecco’s phosphate-buffered saline (D-PBS; Thermo Fisher Scientific, Inc., USA) and extracted three times by intense mixing for 1 min, then left to stand for 5 min in an icebox. The upper aqueous portion was then collected via centrifugation (12,000 × g for 10 min at 4 °C). Eight-week-old GF or gnotobiotic mice were sacrificed, and their colons were opened using a longitudinal incision. The colonic tissue was washed twice in cold Hank’s balanced salt solution (HBSS) and incubated with HBSS containing 30 mM EDTA and 1 mM dithiothreitol at 37 °C and 5% CO2 for epithelial detachment. After 20 min, the colons were washed with HBSS, and epithelial cells were detached using curbing needles in cold HBSS. Detached epithelial cells were collected via centrifugation (500 g for 5 min at 4 °C) and washed twice with HBSS. Some of the epithelial cells were stained with PI and counted using a cytometer (FACSCalibur, BD Biosciences, USA). The remaining epithelial cells were plated on tissue culture dishes. The remaining epithelial cells were plated on tissue culture dishes.
epithelial cells were added to RIPA buffer (Thermo Fisher Scientific) supplemented with protease inhibitor cocktail (Thermo Fisher Scientific) and homogenised by passing through 20 times. The aqueous portion was collected via centrifugation (15,000 g for 10 min at 4 °C). The aqueous portion was also used to analyse protein expression. The organoids were dissociated using GentleMax (Miltenyi Biotec, Germany) and washed with HBSS. Extraction was then performed in the same manner as that for the CECs. The polyclone concentrations in each extraction were measured using ethane-1,2-dioxy- and trioxide-1,6-diamino hexane as an internal standard. The derivatised sections were incubated with a 1:5:1 split into a GCMS-QP2010 gas chromatography coupled with a mass spectrometer detector (GC-MS; GCMS-QP2010, Shimadzu, Co., Japan). Helium was used as a carrier gas. Samples were analysed using the ZB-5 capillary column (60 m × 0.25 mm, 0.25-µm film thickness, Shimadzu). The injector and source temperatures were 260 °C and 150 °C, respectively. The GC oven was programmed as follows: the starting temperature of 140 °C was increased to 190 °C at a rate of 8 °C/min, increased to 320 °C at a rate of 20 °C/min, and finally held for 5 min. Quantification was performed using selected ion monitoring. The ions were monitored at m/z 117 for acetic acid, m/z 131 for propionic acid, and m/z 173 for 2-EB, m/z 261 for lactic acid, and m/z 289 for succinic acid. The ions were monitored at m/z 117 for acetic acid, m/z 131 for propionic acid, and m/z 173 for 2-EB, m/z 261 for lactic acid, and m/z 289 for succinic acid. The extraction and derivatization rates were standardised using 1,6-diamino hexane and quantified using the corresponding external calibration curves.

Western blotting: Rabbit anti-hyphusine antibody (AB1064, Merck Millipore, USA, 1:5000), mouse anti-β-actin antibody (A1978, Sigma-Aldrich, 1:2500) and mouse anti-actin antibody (017–2455, Fujifilm Wako Pure Chemical Corporation) were used for detection of hyphusine protein expression and as a loading control. Total OXPHOS Rodent WB Antibody Cocktail (ab110413, Abcam, Cambridge, UK; 1:1000) was used for detection of mitochondrial OXPHOS-complex expression and as a loading control. Protein denaturated at 95 °C for 5 min were separated via SDS-PAGE on a 4–20% Mini-PROTEAN® TGX™ Gels (Bio-Lad Laboratories, Inc., USA), then electrophoretically transferred to Immuno-Blot polyvinylidene difluoride membrane (Bio-Lad Laboratories). Protein denaturation was not performed to detect total OXPHOS. Protein was detected using horse- radish peroxidase-labelled secondary antibodies and the enhanced chemilumines- cent reagents. Band intensity was quantified using ImageJ software (version 1.47v, National Institutes of Health, USA).

Inhibition of DHs in vivo: Seven-week-old SPF Iqu/loc mice were administered GC7 (Santa Cruz Biotechnology Inc., CA, USA) of 500 µM in drinking water for 5 days. Mice were injected intraperitoneally with 50 µg EdU (Kanto Chemical) 3 h before sacrifice. Western blotting and EdU assay mentioned above.

Colonic organoid cultures: Colonic crypts were isolated from the C57BL/6J mouse colons. The colonic tissue was washed and incubated in 10 ml of isolation buffer (43.4 mM L-sucrose·54.9 mM D-sorbitol·96.2 mM NaCl·5.6 mM Na2HPO4/NaH2PO4·1 H2O·0.55 M Tris·0.55 M KCl·15 mM MgCl2·250 µM CaCl2·250 µM Na2EDTA·100 µM ascorbic acid·100 µM sodium pyruvate·5 µM antioxidant >98% (Sigma-Aldrich) was solubilised in saline and neutralised to pH 7.4 before storage at −80 °C. The organoids were cultured with standard medium added with stable isotope-labeled precursors (for inhibition of DHs: 15N2-putrescine 2·HCl, 15N2-spermidine 2·HCl or 15N2-spermine 2·HCl with atomic purity >98% (Sigma-Aldrich) was solubilised in saline and neutralised to pH 7.4 before storage at −80 °C. The organoid medium was replaced every 2 days. Inhibition of DHS: GC7 was added to the culture medium to disrupt the tissues through 100-µm nylon-mesh cell strainers (BD Biosciences) and transferred to 48-well (for the putrescine tracing experiment and inhibition of spermidine synthesis) or 24-well plates (for inhibition of DHs). After polymerising the Matrigel, standard medium was overlaid on the gel in each well. For the putrescine tracing experiment, 15N2-putrescine 2-HCl with atomic purity >98% (Sigma-Aldrich) was solubilised in saline and neutralised to pH 7.4 before storage at −80 °C. The organoid medium was replaced every 2 days. Inhibition of DHs: GC7 was added to the culture medium from the 4th to the 6th day after initial culturing the organoids for 3 days without GC7. The average size of each organoid was quantified using ImageJ software. The protein was extracted RIPA buffer (Thermo Fisher Scientific) supplemented with protease inhibitor cocktail (Thermo Fisher Scientific) by sonication.

Preparation of immune cells: Colonic tissues were treated with HBSS containing 1 mM diithiothreitol, 20 mM ethylenediaminetetraacetic acid (EDTA) and 12.5 mM HEPEs at 37 °C for 30 min on a stirrer. The tissues were then minced and dissociated in RPMI-1640 containing 0.5 mg ml−1 collagenase (Wako), 0.125 mg ml−1 D-Nase I, 2% new-born calf serum, 100 µg ml−1 penicillin, 100 µg ml−1 strepto- mycin, and 20 mM HEPEs (2×) at 37 °C for 30 min to obtain single-cell suspensions. Single splenocyte suspensions were prepared by mechanically disrupting the tissues through 100-µm nylon-mesh cell strainers (BD Biosciences) in 2 ml of media. These preparations were then subjected to flow cytometry analysis using the LSR II flow cytometer (BD Biosciences).

Flow cytometry: Leucocytes were incubated with anti-CD16/CD32 antibody (AT-10, BioLegend, USA) to block the Fc receptors. Macrophages from eLP and BMDMs were surface stained with BV 510-conjugated anti-CD45 antibody (30-F11, BioLegend), FITC-conjugated anti-CD11b antibody (M1/70, Thermo Fisher Scientific), BV595-conjugated anti-CD11c antibody (HL3, BD Biosciences, PerCP/Cy5.5-conjugated anti-F4/80 antibody (BM8, BioLegend), PE-conjugated anti-CD19 antibody (R-70019; BD Biosciences), APC-conjugated anti-Ly6C antibody (AL-21, BD Biosciences). Macrophages from spleen were surface stained with BV 510-conjugated anti-CD45 antibody (30-F11, BioLegend), PE-conjugated anti-CD16/CD32 antibody (AT-10, BioLegend, USA) to block the Fc receptors, and then stained with BV 510-conjugated anti-CD45 antibody (30-F11, BioLegend), PE conjugated anti-CD19 antibody (R-70019; BD Biosciences).
anti-CD11b antibody (M1/70, BioLegend), PerCP/Cy5.5-conjugated anti-F4/80 antibody (BM6, BioLegend), Alexa Fluor 488-conjugated anti-CXCR1 antibody (R&D Systems, USA) and APC-conjugated anti-Ly6C antibody (AL-21, BD Biosciences). Intracellular staining of NO2 and Arg1 was performed via surface staining with BV 510-conjugated anti-CD45 antibody (30-F11, BioLegend), BVU737-conjugated CD11b antibody (M1/70 BD Biosciences), BVU395-conjugated anti-CD11c antibody (HL3, BD Biosciences) and APC-conjugated anti-F4/80 antibody (BM8, BioLegend). Cells were fixed and permeabilised using the Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific) and stained with PE-conjugated anti-NO2 antibody (CXNFT Thermo Fisher Scientific) and FITC-conjugated anti-Arg1 antibody (R&D Systems). CD4+Foxp3+ cells were surface stained with anti-CD45 antibody (30-F11, BD Biosciences), BVU737-conjugated, anti-CD3e antibody (145–2C11, BD Biosciences) and BV510-conjugated, anti-CD14 (RM4–5, BD Biosciences). After surface staining, cells were fixed and permeabilised using the Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific) and stained with PerCP-Cy5.5-conjugated anti-CD25 antibody (FJK-16s, Thermo Fisher Scientific). Dead cells were detected using Fixable Viability Stain 780 (BD Biosciences). The cells were analysed using the LSR II flow cytometer (BD Biosciences). Gating strategies used for flow cytometry analysis were shown in Supplementary Fig. 8.

**BMDMs culturing.** Bone marrow cells were isolated from the femurs and tibias of the C57BL/6 J male mice. Cells were seeded in 12-well plates at 1 × 106 cells ml−1 in complete RPMI medium (RPMI-1640 medium supplemented with 10% fetal bovine serum, 10 mM HEPES, 20 µM GlutaMAX I (Fisher Scientific), 50 µM 2-mercaptoethanol (Wako), 100 U ml−1 penicillin, 100 µg ml−1 streptomycin) supplemented with 20 ng ml−1 macrophage colony-stimulating factor (BioLegend). The medium in the wells was replaced with fresh medium on day 3. On day 6, BMDMs were harvested and analysed using flow cytometry as mentioned above. The putrescine group was treated with 100 µM putrescine from day 1 to day 6. For the putrescine tracing experiment, 15N2-putrescine 2·HCl with atomic purity >98% (Sigma-Aldrich) was solubilised in saline and neutralised to pH 7.0. For the putrescine tracing experiment: On day 6, BMDMs were cultured with 100 µM stable isotope-labelled putrescine for 24 h. Then, BMDMs were washed and lysed with RIPA buffer, and enzyme enrichment was analysed via GC-MS. For the evaluation of OXPHOS and hyp-eIF5A: From day 6 to day 9, BMDMs were cultured with macrophage colony-stimulating factor in the presence or absence of DFMO (1 mM) and putrescine (100 µM). Then, BMDMs were washed and lysed with RIPA buffer supplemented with protease inhibitor cocktail (Thermo Fisher Scientific). The lysate was used for Western blotting analysis of hyp-eIF5A and OXPHOS.

**DSS-induced experimental colitis.** Eight-week-old mice treated with the SK930 or WT strains were treated with 2.0% DSS (MW 5000, Wako) aqueous solution sterilised through a 0.22-µm filter. To induce colitis, mice were treated with 2.0% DSS aqueous solution for 6 days followed by normal drinking water for 3 days, during which we observed survival rate and disease activity index (DAI)

**Histological scoring.** Colon tissue samples were obtained from 1 cm above the crypt architecture (normal

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Author contributions

A.N. performed the gnotobiote experiments, analysis of polyamine concentrations, and in vitro experiments using BMDMs and intestinal organoids; S. Ku. Designed and pre-
experiments; S.F. performed metabolome analysis; D.T., Y.N. and Y.F. supported flow cytometry and the in vitro experiments; S. Ki. performed histological examination; W.O. performed the intestinal organoids experiments, and OXPHOS western blotting. M.O. helped in vitro experiments using BMDMs. Y.S. and S.S. supported autophagy experiments. A.K. performed immunohistochemical analysis of autophagy; A.N., M.M. and K.H. designed the study and interpreted the data; A.N. and M.M. wrote the manuscript; K.H. critically revised the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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