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Preventive effects of kefir on colon tumor development in Wistar rats: gut microbiota critical role

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Original Article

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Abstract

To clarify the effects of kefir in critical periods of development in adult diseases, we study the effects of kefir intake during early life on gut microbiota and prevention of colorectal carcinogenesis in adulthood. Lactating Wistar rats were divided into three groups: control (C), kefir lactation (KL), and kefir puberty (KP) groups. The C and KP groups received 1 mL of water/day; KL dams received kefir milk daily (10^8 CFU/mL) during lactation. After weaning (postnatal day 21), KP pups received kefir treatment until 60 days. At 67 days old, colorectal carcinogenesis was induced through intraperitoneal injection of 1, 2-dimethylhydrazine. The gut microbiota composition were analyzed by 16S rRNA gene sequencing and DESeq2 (differential abundance method), revealing significant differences in bacterial abundances between the kefir consumption periods. Maternal kefir intake strong anticancer power, suppressed tumors in adult offspring and reduced the relative risk of offspring tumor development. The gut microbiota in cecal samples of the KL group was enriched with *Lactobacillus*, *Romboutsia*, and *Blautia*. In contrast, control animals were enriched with *Acinetobacter*. The administration of kefir during critical periods of development, with emphasis on lactation, affected the gut microbial community structure to promote host benefits. Pearson analysis indicated positive correlation between tumor number with IL-1 levels. Therefore, the probiotic fermented food intake in early life may be effective as chemopreventive potential against colon tumor development, especially in lactation period.

Introduction

Kefir, a probiotic fermented milk produced from kefir grains, contains a complex mixture of bacteria, yeasts in a matrix of proteins, and polysaccharides[1]. Kefir exerts anticancer properties through different mechanisms, including participation in several biological processes, such as antioxidant, apoptosis and proliferation, secretion of cytokines, suppression of the Th2 immune response and activation of the Th1 immune response[2–4]. For this reason, fermented dairy foods have gained evidence in the cancer context[5].

Cancer represents a leading cause of death globally and is a public health problem worldwide. According to data published by the International Agency for Research on Cancer, there were an estimated 19.3 million new cases and 10 million cancer deaths worldwide in 2020. Colorectal cancer ranks third in neoplasia incidence and second among causes of mortality for both sexes[6]. Dietary factors have an impact on cancer risk[7]. In this sense, clinical and preclinical evidence has shown that probiotics have antimutagenic and immunomodulatory activity and can act in the prevention and treatment of colorectal cancer[8,9].

In addition to these factors, the gut microbiota influences the development of colon tumors[10]. The host colonization starts in early life, and the development of the microbiota is influenced by diverse conditions, such as the mode of delivery, type of feeding, genetic factors, diet, mother's age and metabolic status, and antibiotic use[11]. Breast milk is an essential factor for the development and maturation of an infant's microbiota[12]. Bioactive milk components and the breast milk microbiome are transferred by breastfeeding and have an impact on the infant gut microbiota[13]. Due to the difficulties of follow-up and evaluation, we do not fully know the effects of breastfeeding on the long-term composition of the gut microbiota of the progeny and how this can impact the development of diseases in adulthood. The composition of the offspring's gut

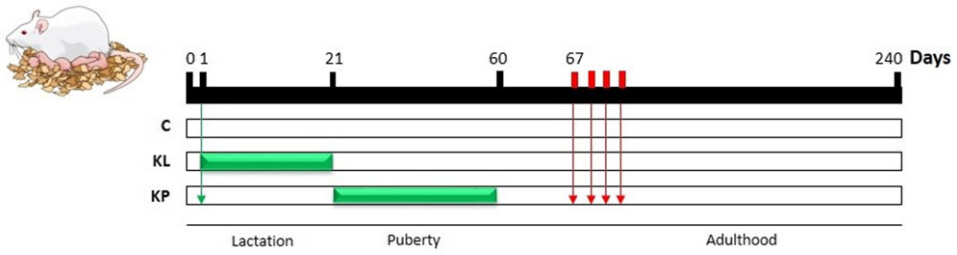

Figure 1. Experimental protocol. On postnatal day (PN) 1, litters from both groups of dams were adjusted to ten pups. The groups of dams were placed on kefir treatment (KL, 10^8 CFU/mL, green indicated) or water (C) for the 21 days of the lactation period. The Kefir puberty (KP) group litters received the kefir treatment postweaning up to PN60 and were then maintained on commercial chow until PN240. Offspring (C, KL, and KP) received 1,2-dimethylhydrazine (DMH, red arrows) at PN 67, 69, 73, and 75 for colon cancer induction. After 24 weeks, the animals were euthanized, the cecal content was collected, and gastrointestinal tissues were evaluated for the presence of tumors. Colony forming unit (CFU).

microbiota is dependent on the maternal diet and postweaning infant diet and can be altered by changing the environment at the postnatal stage[14].

Studies have shown that the way in which certain environmental factors, such as nutritional factors, present themselves in critical periods of development, including lactation and puberty, can influence human biology and health in the long term, reinforcing the concept of "developmental origins of health and disease" (DOHaD)[15]. These critical periods are crucial for developmental plasticity, where epigenetic responses to environmental changes may lead to transgenerational transmission[16]. Thus, lactation and puberty are windows of opportunity for intervention to prevent and reduce the risk of several diseases in adult life, including colorectal cancer. In this way, we investigated the long-term effects of kefir consumption during lactation by the mother or at puberty by the offspring on the adult gut microbiota in colorectal cancer-induced rats.

Materials and methods

Experimental design and procedures

Three-month-old Wistar rats (200–250 g) were obtained from the Center of Reproduction Biology of the Federal University of Juiz de Fora, Minas Gerais, Brazil. The study was approved by the Ethics Committee for Animal Handling of the Federal University of Juiz de Fora at Minas Gerais, Brazil (protocol no. 21/2016) and conformed to the guidelines of the Brazilian Council for Control of Animal Experimentation (CONCEA, Brazil). All animals were housed in plastic cages under controlled conditions of humidity (44–65%), light (12-h light/dark cycle), and temperature (22 ± 2°C).

To evaluate the effects of kefir intake during lactation or puberty, a day after the birth of the puppies, the litters were adjusted to ten male pups for each dam. Then, lactating rats with their offspring were randomized using a computer based random order generator, divided into three groups: control (C, $n = 7$); kefir lactation (KL, $n = 8$); and kefir puberty (KP, $n = 7$). To avoid litter effects, male pups from different litters were used in this study. During lactation, dams in the C and KP groups were given 1 mL of filtered water by oral gavage once per day. For the KL group, the dams were given 1 mL of kefir milk (10^8 colony forming units (CFU)/mL) by gavage once per day. After weaning (21 days), KP pups received kefir treatment by gavage until 60 days old (Fig 1a). The offspring of the KP group received an initial volume of 0.2 mL (at weaning), which was progressively increased to 1 mL on the 40th day of life. In addition to their respective treatments, all the animals were allowed free access to standard rodent chow (Nuvilab®, Paraná, Brazil) and drinking water.

At 67 days old, colorectal carcinogenesis was induced. All animals received an intraperitoneal injection of 1,2-dimethylhydrazine (DMH, Sigma–Aldrich, St Louis, MO, USA) at a dose rate of 40 mg/kg body weight twice weekly for 2 consecutive weeks. DMH was freshly prepared and dissolved in 0.9% saline solution containing 1 mM EDTA and 10 mM sodium citrate, pH 8. At 240 days old, equivalent to 24 weeks after the last DMH injection, the animals were euthanized with a lethal dose of ketamine (90 mg/kg) and xylazine (10 mg/kg). After euthanasia, the colon was washed in saline solution and opened along the mesenteric margin. The number, incidence, and location of tumors were assessed, as well as, colon length and weight.

Preparation and analysis of kefir milk

The kefir grains used in the study were obtained from the Department of Nutrition and Health, Federal University of Viçosa, Minas Gerais, Brazil. They were prepared with pasteurized whole milk (Benfica®, Juiz de Fora, Brazil) according to a previous study[17]. In summary, in a glass container, 10 g of kefir grain was inoculated into 100 mL of milk and incubated at 25°C for 24 h. After that period, the grains of the fermented milk were separated. The procedure was repeated daily to obtain fresh kefir during the treatment period. The fermented beverage was analyzed for microbial composition by next-generation sequencing and showed a high prevalence of *Lactococcus* and *Lactobacillus* among the bacterial groups and *Aspergillus* and *Cordyceps* from the fungal community, as previously described[18]. The counts of lactic acid bacteria, yeasts and evaluation of the pH were performed periodically throughout the treatment and were kept at 10^8 CFU/mL, 10^6 CFU/mL and 3.9–4.1, respectively, thus meeting the standards proposed by the international body Codex Alimentarius[19].

Microbiota analysis using 16S rRNA high-throughput sequencing and bioinformatics

Samples were collected directly from the cecum in sterile tubes and randomly chosen using a random number generator from the C ($n = 5$), KL ($n = 5$), and KP ($n = 5$) groups for gut microbiota analysis. DNA was isolated from the samples using the MagaZorb® DNA Mini-Prep Kit (Promega, Madison, WI, USA). 16S rRNA high-throughput sequencing was performed for each sample by GenOne Biotechnologies enterprise (Rio de Janeiro, Brazil).

The V3-V4 region of the 16S rRNA bacterial gene of the DNA samples was amplified using the 341F (5'-CCTAYGGG

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RBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') primers. All PCRs were carried out with Phusion® High Fidelity PCR MasterMix (New England Biolabs, USA). The PCR products were purified with a Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using the NEBNext® UltraTM DNA Library Prep Kit for Illumina, following the manufacturer's recommendations, and index codes were added. The quantity and quality of the libraries were assessed on a Qubit® 2.0 Fluorometer (Thermo Scientific, USA) and Agilent Bioanalyzer 2100 system. Sequencing was carried out on the MiSeq (Illumina, USA) platform (2x 250 bp), as suggested by the Earth Microbiome Project[20]. Amplicon sequence variants (ASVs) were inferred using the DADA2 (Divisive Amplicon Denoising Algorithm 2) method[21], and taxonomy was assigned using a naive Bayesian classifier method against SILVA database release 132[22]. Diversity and phylogenetic analyses were performed with the aid of several R packages, including phyloseq[23], vegan[24], DECIPHER[25], phangorn[26] and ggplot2[27]. All the plots (taxonomy, alpha and beta diversity, differential abundance) were carried out with ggplot2 implemented on R. We used DESeq2 to identify differentially abundant ASVs in pairwise comparisons between the groups[28].

The sequence data were submitted to the GenBank database and are accessible under Bioproject PRJNA672176.

Colonic and inflammatory biomarkers and correlation analysis

For the measurement of cytokine levels in colonic tissues, 100 mg of tissue (medial colon) from each animal was homogenized in 1 mL of PBS buffer containing 0.05% Tween 20, 0.5% bovine serum albumin, protease inhibitors (0.01 mM EDTA), and 20 IU aprotinin A, using a tissue homogenizer. The resulting homogenate was centrifuged at 13,500 r.p.m. for 20 min at 4°C, and the supernatant was used for cytokine quantification. Cytokines were measured using ELISA kits (PeproTech Inc., now moved to Thermo Fisher, Waltham, MA, USA) – Interleukin (IL)-1β (Catalog # 900-M91), IL-6 (Catalog # 900-M86), interferon-γ (Catalog # 900-K109K), and tumor necrosis factor (TNF, Catalog # 900-K73K), following the manufacturer's instructions, and the results were expressed as pg/mL. Total nitric oxide (NO) was evaluated through the Griess reaction[29]. Pearson's correlation coefficient was used to analyze the correlation between microbiota, colonic and inflammatory biomarkers, and visualization was made in the form of a heat map.

Statistical analyses

The calculation of sample size was performed by power analysis using statistical software available online (http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html), considering a statistical power of 0.8 and a type 1 error rate. The data were analyzed by the statistical program GraphPad Prism version 8 (GraphPad Software, Inc., La Jolla, CA, USA) and are expressed as the mean ± standard error of the mean (SEM). Data normality was evaluated by the Kolmogorov–Smirnov test. For parametric analysis, one-way ANOVA followed by Newman–Keuls posttest determined differences between groups, and the relative risk (RR) and 95% confidence interval (CI) calculations were performed using the Koopman asymptotic score method. Correlations were identified by Pearson's correlation coefficient. The differences were considered statistically significant at $p < 0.05$.

Statistical analysis was also performed on the microbiota data. To evaluate alpha diversity differences between groups, the Wilcoxon test was carried out. To test for differences in microbial community composition (PCoA), permutational multivariate analysis of variance on UniFrac distances was used.

Table 1. Nutritional and colon characteristics of pups

	C	KL	KP
Body weight (g)			
21 days old	27.30 ± 0.44	33.99 ± 0.82***	27.59 ± 0.16
60 days old	199.2 ± 3.80	198.4 ± 3.91	193.1 ± 3.90
180 days old	327.5 ± 7.65	339.9 ± 10.58	329.7 ± 5.28
240 days old	331.0 ± 12.21	355.1 ± 12.08	345.1 ± 6.88
Adipose tissue (g)	10.16 ± 0.69	12.85 ± 1.09	11.18 ± 0.68
Glycemia (mg/dL)	120.5 ± 2.84	107.9 ± 3.79	115.0 ± 5.34
Colon length (cm)	20.13 ± 0.38	20.93 ± 0.49	20.56 ± 0.58
Colon weight (g)	2.24 ± 0.20	2.04 ± 0.08	2.38 ± 0.30
Number of tumors	0.571 ± 0.20	0 ± 0*	0.428 ± 0.20

The results are expressed as the mean ± SEM ($n = 7$–8), *$p < 0.05$ vs. C, ***$p < 0.001$ vs. C and KP. Control (C), kefir lactation (KL), kefir puberty (KP).

Results

Maternal kefir intake prevents the development of tumors in offspring

KL offspring presented an increase in body weight at 21 days old, which did not change their body weight and adipose tissue in adulthood. No significant difference was observed in chow consumption between the groups (not shown). At 240 days old, offspring showed no change in glycemia, colon length, and colon weight (Table 1). Animals whose mothers consumed kefir during the lactation period did not develop colon tumors (Supplementary Fig S1), as evidenced by the significant decrease in tumor number compared to that of the control group ($p = 0.027$), as shown in Table 1. Maternal kefir intake resulted in a reduced relative risk (RR) of offspring tumor development (RR = 3.333, 95% CI = 1.282 to 7.700; $p = 0.018$). Photos of the colon (one representative colon from each group) are shown in Figure 2.

16S rRNA sequencing data

The sequencing of the DNA samples extracted from the cecal contents resulted in 609,011 high-quality sequences with an average of 40,600 sequences per sample. Reads were clustered into 1,716 ASVs. Rarefaction curves indicated that sampling provided sufficient ASV coverage to accurately describe the bacterial composition of each sample, as it could be seen in Supplementary Fig S2.

Maternal kefir intake increases the richness and diversity of the offspring gut microbiota

The richness and diversity of the gut microbiota of the progeny were evaluated. The number of observed ASVs did not differed statistically between control and groups KL and KP. However, the observed ASVs was higher in the KL group, whose dams received kefir during lactation, than in the puberty alone group ($p = 0.021$) (Fig 3). The Simpson index was also greater in the KL group,

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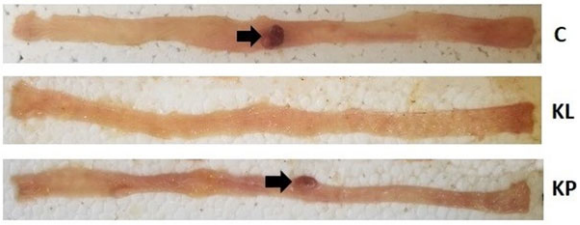

Figure 2. Maternal kefir intake prevents colon tumor development. Macroscopic view of the colons of each group, control (C), Kefir lactation (KL), and Kefir puberty (KP).

although the difference was not significant (Fig 3). In contrast, the overall microbial community structures (beta diversity) differed significantly ($r2 = 0.499$; $p = 0.001$ by ADONIS test, cluster centroids are shown in Supplementary Fig S3) between the groups, as shown in PCoA using weighted UniFrac distance (Fig 3). However, it is possible to note that the microbiota from the C and KP groups were more related to each other when compared with that of the KL group.

The critical period of diet intervention affects the gut microbiota in adulthood

The phyla Bacteroidetes, Firmicutes, and Proteobacteria dominated the gut microbiota of the animals (Fig 4). According to the taxonomic data, the microbiota composition of control animals (C) is in some ways more similar to the ones that received kefir during puberty (KP) and differed significantly from the animals whose dams received kefir during lactation (KL). Bacteroidetes were more abundant in the C and KP groups than in the KL group, while the opposite was observed for Firmicutes (Fig 4). The Proteobacteria abundance was higher in the control animals. The results showed that kefir administration in early life modified the microbiota of the animals in adulthood. The overall microbial composition for each sample at the family and genus levels is shown in Supplementary Fig S4 and S5. We observed a reduction in the abundances of the genera *Acinetobacter*, *Prevotella_9*, *Prevotellaceae-NK3B31*, and *Prevotellaceae_UCG-001* and an increase in the abundances of *Lactobacillus* and *Lachnospiraceae_NK4A136_group* in the intestinal microbiota of KL animals. A rise in the abundance of the genus *Lachnospiraceae_NK4A136_group* was also observed in the KP group (Supplementary Fig S5). Differential abundance using DESeq2 was carried out to verify significant ASV differences between the groups. Fifteen ASVs were differentially abundant between the C and KL groups, with enrichment in ASVs associated with *Lactobacillus*, *Ruminococcaceae_UCG-14*, and *Coprococcus_2* in the KL group (Fig 5). *Acinetobacter* and members of Prevotellaceae were enriched in the control group. We confirmed that the genera *Lactobacillus*, *Romboutsia*, *Blautia*, and *Lachnospiraceae_UCG-004* were significantly ($p < 0.001$) associated with kefir maternal intake during lactation, while members of the *Prevotellaceae* family and *Bacteroides* were enriched in the animals that received kefir in puberty (Fig 5).

Tumor numbers have a strong correlation with IL-1

To better understand the relationship between gut microbiota and colon carcinogenesis, we conducted a correlation analysis between genus-level microorganisms and key biomarkers. As shown in Fig 6, the heat map indicated that the *Acinetobacter* genus was positively related to the pro-inflammatory cytokine TNF (Supplementary Table S1). *Lactobacillus* abundance was positively related to colon length, showing its trophic effects on gut mucosa. By contrast, NO which were negatively associated with the colon length. There is a strong correlation ($r = 0.911$; $p = 0.002$) between tumor number and IL-1 level, confirming the important relationship between inflammation and the tumor development.

Discussion

In this study, we show for the first time that kefir intake by mothers during lactation or by offspring at puberty produced distinct changes in the richness and composition of the gut microbiota of adult offspring induced by colorectal cancer. The administration of kefir in dams during lactation was associated with the nondevelopment of tumors and an increased microbial richness and relative abundance of genera associated with gut health in male offspring. However, administration of kefir up to puberty in pups did not have such significant effects on chemically induced tumor development and gut microbiota composition outcomes in offspring. These data together demonstrate the importance of lactation for the establishment of the intestinal microbiota and intestinal health of the progeny.

Maternal kefir intake during lactation prevented tumor development in adult offspring. Kefir anticancer properties were previously reported[4], although in this study, we investigated the effects of its use in early life on the development of diseases in adults. Different environmental exposures can result in epigenetic modifications that act by modulating chromatin structure and gene regulation that do not involve changes to the underlying DNA sequence[29]. Distinct epigenetic profiles can be retained throughout the lifespan and may be inherited with transgenerational effects[30]. In this sense, epigenetic regulation has been reported to contribute to cancer development and progression[31]. Thus, exposure to environmental factors, such as diet during early life, may lead to favorable or unfavorable consequences, impacting the risk and prevention of diseases in the progeny[32].

Unlike previous studies[34,35], our group has studied the effects of kefir intake during critical periods of development on adult offspring. Our group previously demonstrated that kefir treatment during lactation and continued during puberty had an impact on colorectal carcinogenesis via a reduction in mucosa inflammation, reversing the effects of neonatal overfeeding [36]. Here, we show that the period of lactation is a determinant period of these beneficial effects; that is, the maternal kefir intake during lactation is able to modulate the gut microbiota and program adult offspring to prevent the development of colorectal cancer. It is known that the establishment of the intestinal microbiota occurs early in life[37,38] and that this initial microbiota will affect the microbial core in adulthood, potentially leading to more pronounced preventive effects; which was confirmed in our study.

High bacterial richness was a marker of cancer protection in our study. A species-rich gut ecosystem is more robust against environmental influence, and these microbial communities are resilient and more resistant to change [39], which seems to have occurred in the KL group. These animals did not develop colon tumors during the follow-up. A low bacterial richness was associated with an inflammatory phenotype in obese individuals[40], and intestinal dysbiosis and increased intestinal permeability are factors that contribute to inflammation[41], creating a favorable microenvironment for neoplastic development[42]. In addition to the change in structure, we also observed long-term alterations in

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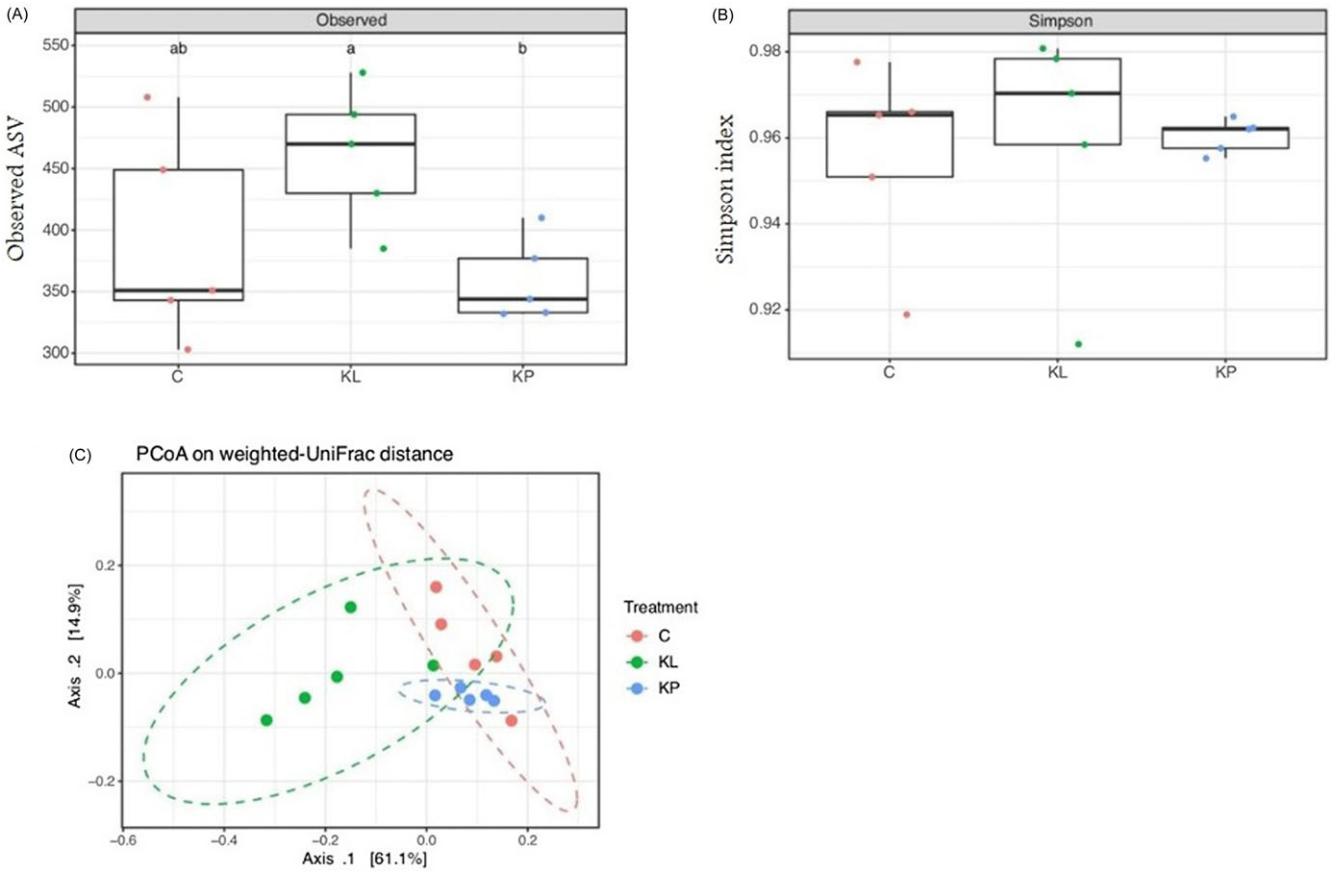

Figure 3. Maternal kefir intake improves gut microbiota richness in adult offspring with DMH-induced colon carcinogenesis. Analysis of observed ASV, alpha diversity assessed by the Simpson index, and beta diversity principal coordinates analysis (PCoA) plot using weighted uniFrac distance highlighting differences between microbial communities ($p = 0.002$ by ADONIS test) in the groups. Control (C), Kefir lactation (KL), Kefir puberty (KP).

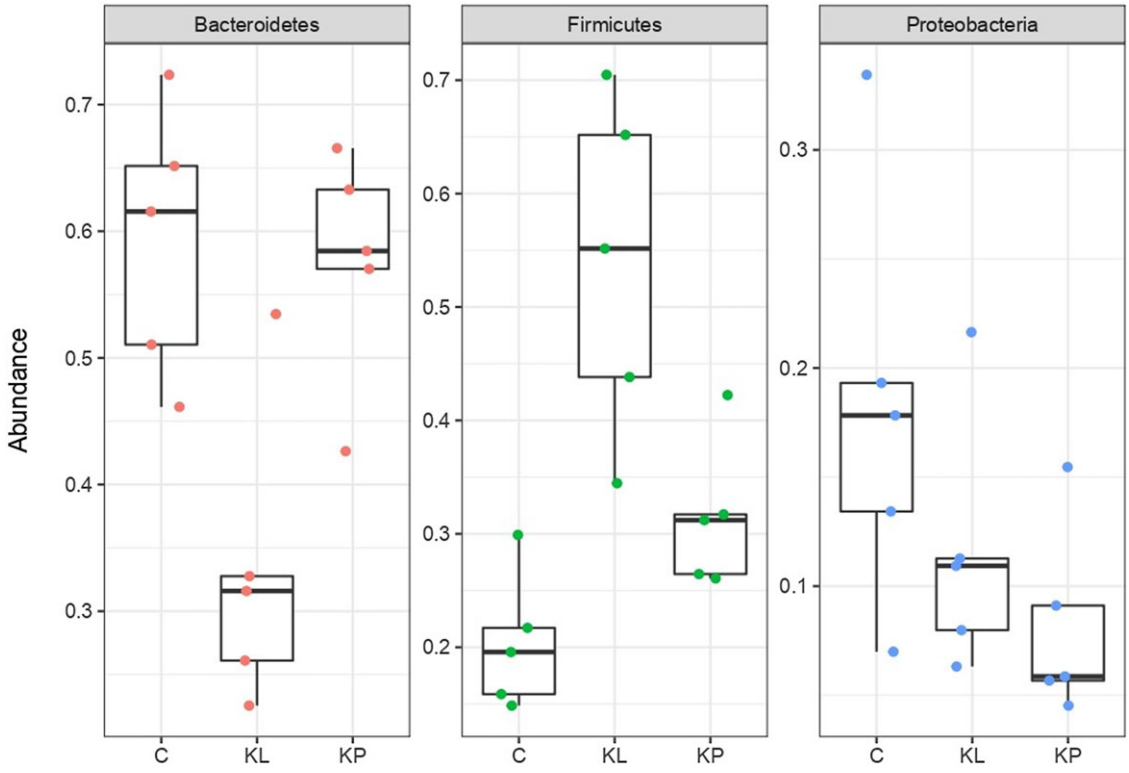

Figure 4. Kefir intake affects the bacterial abundance in the model of colorectal cancer. Boxplots of the relative abundances of bacterial phyla in the gut microbiota of each group: control (C), Kefir lactation (KL), and Kefir puberty (KP). Data are presented as the median, minimum, and maximum values, $n = 5$.

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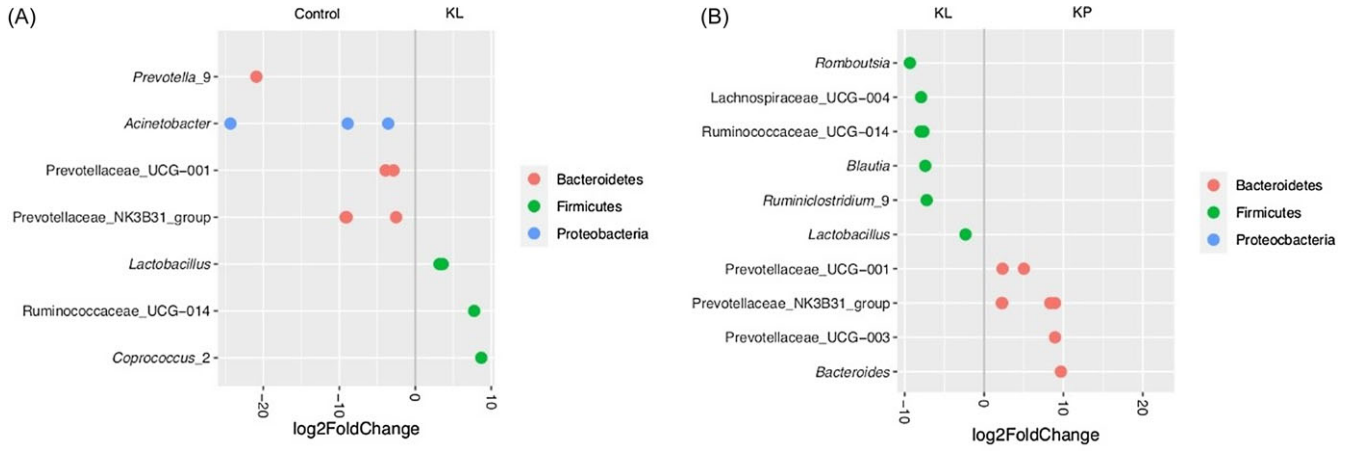

Figure 5. The gut microbiota of offspring whose mothers received kefir during lactation is enriched with butyrogenic and lactic bacteria. Plot showing the enrichment of genera with significant differential abundance between the control and KL groups, and the KL and KP groups, as detected and filtered by DESeq2. Control (C), Kefir lactation (KL), Kefir puberty (KP).

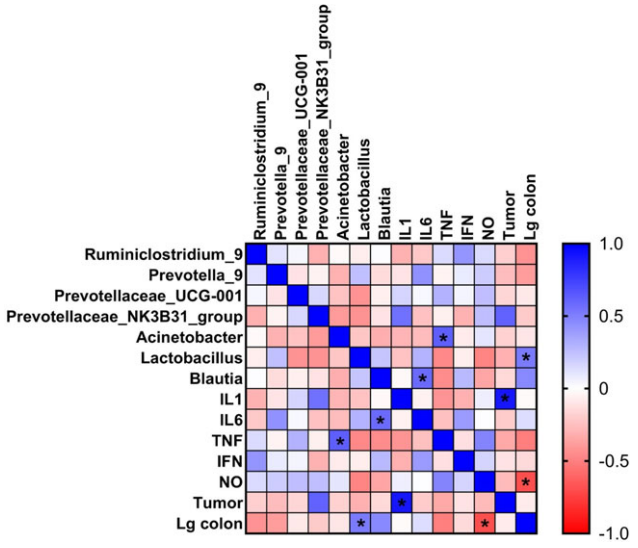

Figure 6. Correlation between microorganisms (genus level), colonic and inflammatory biomarkers. Blue is positive correlation, and red is negative correlation, $^{*}p < 0.05$.

the composition of the microbiota between groups. Alterations in the gut microbiota confer a predisposition to certain malignancies and influence the response to a variety of cancer therapies[43]. The lower diversity may explain the high incidences of autoimmune and inflammatory disorders[44]. It is known that the microbiota is relatively stable after the first years of life [45]; thus, exposure to modulating factors, especially in early periods, can promote alterations that have repercussions in later life.

We found an increase in the *Lachnospiraceae_NK4A136_group* in the cecal microbiota of animals that received kefir for one of the periods, lactation or puberty. This genus is a butyrate producer and has been shown to protect against inflammation [46]. In contrast, control animals presented an increase in the relative abundance of *Acinetobacter* (Proteobacteria phylum); species in this genus, such as *Acinetobacter baumannii*, are opportunistic pathogens [47] and are frequently isolated from cancer patients [48]. The association of the genera *Lactobacillus*, *Romboutsia*, and *Blautia* with kefir maternal intake during lactation may be a good indication of the advantage of this period over the postweaning period for the establishment of the adult gut microbiota and colon cancer prevention. *Lactobacillus* was the second most abundant bacterial genus found when kefir milk was used to treat animals[18]. Thus, it is possible that these animals received a greater amount of these bacteria via breast milk during lactation. It has already been shown in animal[49] and human studies[50] that breast milk is capable of modulating the intestinal microbiota of the progeny, with breastfeeding being fundamental for the establishment of the gut microbiota, especially colonization by *Lactobacillus*[51].

Enrichment of the *Romboutsia* and *Blautia* genera in lactation compared to the puberty period was observed. *Romboutsia* has already been identified in the human gut and was reported to be depleted in cancerous mucosa, representing a novel microbial biomarker associated with colorectal cancer[52]. This depletion was also recently observed in pancreatic cancer patients[53]. According to these findings, the KL animals did not develop colon tumors, suggesting an association between *Romboutsia* and a possible protection against cancer. The *Blautia* genus is widely distributed in mammalian feces and intestines, and some species can use carbohydrates as energy sources with the production of acetic acid and long-chain fatty acids, among others[54]. The enrichment of *Blautia* in mice fed fructooligosaccharide [55] and its reduction in the mucosal adherent microbiota of colorectal cancer patients [56] have also been observed.

Here, although the *Blautia* anti-inflammatory effect, we observed a positive correlation with IL-6. Meanwhile, the cytokine IL-1 was significantly correlated with the number of tumors. IL-1 has a significant role in the pathogenesis of colorectal cancer[57], and it can be secreted by tumor cells and induce the recruitment of immunosuppressive cells[58]. Furthermore, as shown in a previous study by the group, the combination of the two critical periods (lactation and puberty) enhanced the anti-inflammatory effects of kefir on the colonic mucosa, leading to a significant reduction in pro-inflammatory cytokines[36]. The results presented here for treatment during independent periods (lactation or puberty) suggest that critical pathways for the anti-tumor immune response are developed in these phases[59,60], with the contribution of several cytokines[61], and should be investigated in future studies.

In summary, the administration of kefir during the critical periods of development affected the gut microbiota community structure to promote host benefits with a reduction in colon tumor

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development. Maternal and postweaning kefir intake produced a distinct change in the richness and composition of the gut microbiota of colon cancer-induced offspring, where the lactation period was superior with respect to the programming of the gut microbiota of the adult offspring. These findings suggest that the maternal use of probiotic fermented food during lactation could be a strategy for colon cancer prevention in progeny, especially when there are associated risk factors.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S2040174424000461.

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Author contribution. **PGAB:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft. **JDM:** Formal analysis, Writing – Original Draft. **TCA:** Investigation, Writing – Review & Editing. **CTS:** Investigation. **GCAA:** Investigation. **ABFM:** Visualization, Writing – Review & Editing. **SCPDL:** Conceptualization, Funding acquisition, Supervision, Writing – Original Draft.

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Competing interests. None.

References

1. Farnworth ER. Kefir - A complex probiotic. *Food Sci Technol Bull Funct Foods*. 2005; 2, 1–17.
2. Bengoa AA, Iraporda C, Garrote GL, Abraham AG. Kefir micro-organisms: their role in grain assembly and health properties of fermented milk. *J Appl Microbiol*. 2019; 126, 686–700.
3. Khoury N, El-Hayek S, Tarras O, El-Sabban M, El-Sibai M, Rizk S. Kefir exhibits anti-proliferative and pro-apoptotic effects on colon adenocarcinoma cells with no significant effects on cell migration and invasion, - proliferative and pro-apoptotic effects on colon adenocarcinoma cells with no significant effects on cell migration and invasion. *Int J Oncol*. 2014; 45, 2117–2127.
4. Sharifi M, Moridnia A, Mortazavi D, Salehi M, Bagheri M, Sheikhi A. Kefir: a powerful probiotics with anticancer properties. *Med Oncol*. 2017; 34, 183.
5. Zhang K, Dai H, Liang W, Zhang L, Deng Z. Fermented dairy foods intake and risk of cancer. *Int J Cancer*. 2019; 144, 2099–2108.
6. Sung H, Ferlay J, Siegel RL, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin*. 2021; 71, 209–249.
7. Steck SE, Murphy EA. Dietary patterns and cancer risk. *Nat Rev Cancer*. 2020; 20, 125–138.
8. Eslami M, Yousefi B, Kokhaei P, *et al.* Importance of probiotics in the prevention and treatment of colorectal cancer. *J Cell Physiol*. 2019; 234, 17127–17143.
9. de Brasiel P.G. A, Dutra Luquetti SCP, do Peluzio MCG, Novaes RD, Gonçalves RV. Preclinical evidence of probiotics in colorectal carcinogenesis: a systematic review. *Dig Dis Sci*. 2020; 65, 3197–3210.
10. Rinninella E, Raoul P, Cintoni M, *et al.* What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*. 2019; 7, 14–22.
11. Milani C, Duranti S, Bottacini F, *et al.* The first microbial colonizers of the human gut: composition, activities, and health implications of the infant gut microbiota. *Microbiol Mol Biol Rev*. 2017; 81, e00036–e00017.
12. Pannaraj PS, Li F, Cerini C, *et al.* Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatr*. 2017; 171, 647–654.
13. Komatsu Y, Kumakura D, Seto N, Izumi H. Dynamic associations of milk components with the infant gut microbiome and fecal metabolites in a mother–infant model by microbiome, NMR metabolomic, and time-series clustering analyses. *Front Nutr*. 2022; 8, 1–13.
14. Edwards CA. Determinants and duration of impact of early gut bacterial colonization. *Ann Nutr Metab*. 2017; 70, 246–250.
15. Barker DJ, Bull AR, Osmond C, Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. *BMJ*. 1990; 301, 259–262.
16. Sookoian S, Gianotti TF, Burgueño AL, Pirola CJ. Fetal metabolic programming and epigenetic modifications: a systems biology approach. *Pediatr Res*. 2013; 73, 531–542.
17. Kim D-H, Chon J-W, Kim H, *et al.* Detection and eenumeration of Lactic Acid Bacteria, Acetic Acid Bacteria and Yeast in kefir grain and milk using quantitative Real-Time PCR. *J Food Saf*. 2015; 35, 102–107.
18. de Brasiel P.G. A, Medeiros JD, Machado ABF, Ferreira MS, do Peluzio MCG, Luquetti SCPD. Microbial community dynamics of fermented kefir beverages changes over time. *Int J Dairy Technol*. 2021; 74, 324–331.
19. World Health Organization/ Food and Agriculture Organization of the United Nations. *Milk and Milk Products*, 2nd ed. Rome; 2011. 244 p.
20. Caporaso JG, Lauber CL, Walters WA. Ultra-high-throughput microbial community analysis on the illumina hiSeq and miSeq platforms. *ISME J*. 2012; 6, 1621–1624.
21. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: high resolution sample inference from illumina amplicon data. *Nat Methods*. 2016; 13, 581–583.
22. Pruesse E, Quast C, Knittel K, Fuchs BM, Glo FO, Ludwig W. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res*. 2007; 35, 7188–7196.
23. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. 2013; 8, e61217.
24. Oksanen J, Blanchet FG, Friendly M, *et al.* Vegan: community ecology package. R package Version 2.5-6 [Internet] 2019, Available from: https://cran.r-project.org/.
25. Wright Erik S. Using DECIPHER v2.0 to analyze big biological sequence data in R. *R J [Internet]*. 2016; 8, 352.
26. Schliep KP. Phangorn: phylogenetic analysis in R. *Bioinformatics [Internet]*. 2011; 27, 592–593.
27. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag; 2016. https://doi.org/10.1007/978-3-319-24277-4_9.
28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014; 15, 550.
29. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*. 2001; 5, 62–71.
30. Bianco-Miotto T, Craig JM, Gasser YP, van Dijk SJ, Ozanne SE. Epigenetics and DOHaD: from basics to birth and beyond. *J Dev Orig Health Dis*. 2017; 8, 513–519.
31. Fitz-James MH, Cavalli G. Molecular mechanisms of transgenerational epigenetic inheritance. *Nat Rev Genet*. 2022; 23, 325–341.
32. Bailey MH, Tokheim C, Porta-Pardo E, *et al.* Comprehensive characterization of cancer driver genes and mutations. *Cell*. 2018; 173, 371–385.e18.
33. Lambrot R, Xu C, Saint-Phar S, *et al.* Low paternal dietary folate alters the mouse sperm epigenome and is associated with negative pregnancy outcomes. *Nat Commun*. 2013; 4, 2889.
34. Zanardi KR, Grancieri M, Silva CW, *et al.* Functional effects of yacon (*Smallanthus sonchifolius*) and kefir on systemic inflammation, antioxidant activity, and intestinal microbiome in rats with induced colorectal cancer. *Food Funct*. 2023; 14, 9000–9017.
35. Zeng X, Jia H, Zhang X, *et al.* Supplementation of kefir ameliorates azoxymethane/dextran sulfate sodium induced colorectal cancer by modulating the gut microbiota. *Food Funct*. 2021; 12, 11641–11655.
36. de Brasiel P.G. A, Luquetti SCPD, Medeiros JD, *et al.* Kefir modulates gut microbiota and reduces DMH-associated colorectal cancer via regulation of intestinal inflammation in adulthood offsprings programmed by neonatal overfeeding. *Food Res Int*. 2022; 152, 110708.

37. Xiao L, Zhao F. Microbial transmission, colonisation and succession: from pregnancy to infancy. *Gut*. 2023; 72, 772–786.
38. Tian M, Li Q, Zheng T, *et al*. Maternal microbe-specific modulation of the offspring microbiome and development during pregnancy and lactation. *Gut Microbes*. 2023; 15, 2206505.
39. Sommer F, Anderson JM, Bharti R, Raes J, Rosenstiel P. The resilience of the intestinal microbiota influences health and disease. *Nat Rev Microbiol*. 2017; 15, 630–638.
40. Le Chatelier E, Nielsen T, Qin J, *et al*. Richness of human gut microbiome correlates with metabolic markers. *Nature*. 2013; 500, 541–546.
41. Stone TW, Mcpherson M, Darlington LG. Obesity and cancer: existing and new hypotheses for a Causal connection. *EBioMedicine*. 2018; 30, 14–28. DOI: 10.1016/j.ebiom.2018.02.022.
42. Cani PD. Gut microbiota-mediated inflammation in obesity: a link with gastrointestinal cancer. *Nat Rev Gastroenterol Hepatol*. 2018; 15, 671–682.
43. Sims TT, El Alam MB, Karpinets TV. Gut microbiome diversity is an independent predictor of survival in cervical cancer patients receiving chemoradiation. *Commun Biol*. 2021; 4, 237.
44. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014; 157, 121–141.
45. Laursen MF. Gut microbiota development: influence of diet from infancy to toddlerhood. *Ann Nutr Metab*. 2021; 77, 21–34.
46. Borton MA, Sabag-Daigle A, Wu J. Chemical and pathogen-induced inflammation disrupt the murine intestinal microbiome. *Microbiome*. 2017; 5, 47.
47. McConnell MJ, Actis L, Pachón J. Acinetobacter baumannii: human infections, factors contributing to pathogenesis and animal models. *FEMS Microbiol Rev*. 2013; 37, 130–155.
48. Wu P, Zhang G, Zhao J, *et al*. Profiling the urinary microbiota in male patients with bladder cancer in China. *Front Cell Infect Microbiol*. 2018; 8, 167. Erratum in: *Front Cell Infect Microbiol*. 2018; 8, 429.
49. Morales-Ferré C, Azagra-Boronat I, Massot-Cladera M, *et al*. Effects of a postbiotic and prebiotic mixture on suckling rats' Microbiota and Immunity. *Nutrients*. 2021; 13, 2975.
50. Padilha M, Danneskiold-Samsøe NB, Brejnrod A. The human milk microbiota is modulated by maternal diet. *Microorganisms*. 2019; 7, 502.
51. Stinson LF. Establishment of the early-life microbiome: a DOHaD perspective. *J Dev Orig Health Dis*. 2020; 11, 201–210.
52. Mangifesta M, Mancabelli L, Milani C, *et al*. Mucosal microbiota of intestinal polyps reveals putative biomarkers of colorectal cancer. *Sci Rep*. 2018; 8, 13974.
53. Kartal E, Schmidt TSB, Molina-Montes E, *et al*. A faecal microbiota signature with high specificity for pancreatic cancer. *Gut*. 2022; 71(7), 1359–1372.
54. Liu X, Mao B, Gu J, *et al*. Blautia —a new functional genus with potential probiotic properties? *Gut Microbes*. 2021; 13, 1875796.
55. Gu J, Cui S, Tang X, *et al*. Effects of fructooligosaccharides (FOS) on the composition of cecal and fecal microbiota and the quantitative detection of FOS-metabolizing bacteria using species-specific primers. *J Sci Food Agric*. 2022; 102, 5301–5311.
56. Chen W, Liu F, Ling Z, Tong X, Xiang C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. In*PLoS One*. (ed. Moschetta A), 2012; 7, e39743, Available from: https://dx.plos.org/10.1371/journal.pone.0039743.
57. Cheng KJ, Mejia Mohammed EH, Khong TL, Mohd Zain S, Thavagnanam S, Ibrahim ZA. IL-1α and colorectal cancer pathogenesis: enthralling candidate for anti-cancer therapy. *Crit Rev Oncol Hematol*. 2021; 163, 103398.
58. Xie J, Zhang Y, Jiang L. Role of Interleukin-1 in the pathogenesis of colorectal cancer: a brief look at anakinra therapy. *Int Immunopharmacol*. 2022; 105, 108577.
59. Hauer J, Fischer U, Borkhardt A. Toward prevention of childhood ALL by early-life immune training. *Blood*. 2021; 138, 1412–1428.
60. Drijvers JM, Sharpe AH, Haigis MC. The effects of age and systemic metabolism on anti-tumor T cell responses. *Elife*. 2020; 9, e62420.
61. Yi M, Li T, Niu M, *et al*. Targeting cytokine and chemokine signaling pathways for cancer therapy. *Signal Transduct Target Ther*. 2024; 9, 176.

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