

REVIEW ARTICLE



Epidemiology and Population Health

Spot-light on microbiota in obesity and cancer

Paolo Marzullo^{1,2}, Silvia Bettini³, Davide Menafra⁴, Sara Aprano⁴, Giovanna Muscogiuri⁴✉, Luigi Barrea⁴, Silvia Savastano⁴, Annamaria Colao^{4,5} and on behalf of the Obesity Programs of nutrition, Education, Research and Assessment (OPERA) group*

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Over the last few years, the complexity and diversity of gut microbiota within and across individuals has been detailed in relation to human health. Further, understanding of the bidirectional association between gut microbiota and metabolic disorders has highlighted a complimentary, yet crucial role for microbiota in the onset and progression of obesity-related cancers. While strategies for cancer prevention and cure are known to work efficiently when supported by healthy diet and lifestyle choices and physical activity, emerging evidence suggests that the complex interplay relating microbiota both to neoplastic and metabolic diseases could aid strategies for cancer treatment and outcomes. This review will explore the experimental and clinical grounds supporting the functional role of gut microbiota in the pathophysiology and progression of cancers in relation to obesity and its metabolic correlates. Therapeutic approaches aiding microbiota restoration in connection with cancer treatments will be discussed.

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INTRODUCTION

Noncommunicable diseases (NCDs) are responsible for over 70% of all deaths every year [1]. NCDs mainly include obesity and metabolic disorders, cardiovascular, and cerebrovascular diseases, as well as cancers. Overweight and obesity are estimated to cause 4.5 million deaths annually, and they significantly contribute to the cancer burden in North America, Europe, and the Middle East [2]. Indeed, an association between obesity and increased risk of cancer has long been recognized [3] and, according to an eminent US-based study, the proportion of cancer-related death attributed to obesity is 14% for men and 20% for woman [4]. Moreover, a dose- and time-response relationship links obesity to cancer risk [2, 4]. Growing awareness on the intricate relationship linking obesity and cancer to changes in microbiota has improved our understanding of the underlying causal mechanisms, and prompted investigations on microbiota manipulation to aid antineoplastic treatments. This review aims to explore the main findings supporting a functional role for gut microbiota in the pathophysiology and progression of cancers, with a special focus on obesity-associated neoplasms. Insights on therapeutic approaches aiding microbiota restoration in connection with cancer treatment will also be discussed.

OBESITY-RELATED LOW-GRADE INFLAMMATION AND CANCER RISK

It is recognized that obesity increases susceptibility for 13 different cancers, with a potential association with 3 other tumors (Table 1) [5].

The underlying mechanisms are multiple and include pro-inflammatory processes generated by excessive white adipose tissue (WAT) expansion, which stimulates the secretion of cytokines and chemotactic factors [6]. These include platelet-derived growth factor, transforming growth factor- β , monocyte chemoattractant protein-1, interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α . Such pro-inflammatory mediators can activate endothelial precursor cells, immune cells and preadipocytes accompanying macrophage infiltration in stromal tissue and vascular bed of AT [6]. In the absence of adequate neo-angiogenic support, AT undergoes hypoxia with overexpression of hypoxia-induced factor- α , adipocyte stress, and death. The inherent phenotypical switch in macrophage polarization, from the anti-inflammatory M2 to the pro-inflammatory M1 conformation, engages a process of phagocytosis of dead adipocytes in the context of a circle configuration termed crown-like structure [7, 8]. Lipolytic release of free fatty acids (FFAs) from entrapped adipocytes can activate toll-like receptor-4 on the macrophage plasma membrane and increase nuclear factor kappa B expression through activation of pro-inflammatory genes, like those coding for TNF- α , IL-1 β , and cyclooxygenase-2 (COX-2) [9]. FFAs release is further stimulated by TNF- α and other cytokines sustaining inflammation and, in conjunction with angiogenesis, production of extracellular matrix. Leptin, a cognate product of adipocytes, is also capable of pro-inflammatory, proliferative, and proangiogenic actions through stimulation of TNF- α and IL-6 production. Conversely, the insulin-sensitizing hormone adiponectin is

¹Department of Translational Medicine, Università del Piemonte Orientale, Novara, Italy. ²Laboratory of Metabolic Research, IRCCS Istituto Auxologico Italiano, Piancavallo, Verbania, Italy. ³Department of Medicine, University of Padova, Padova, Italy. ⁴Department of Clinical Medicine and Surgery, Endocrinology Unit, University Faculty of Medicine Federico II of Naples, Naples, Italy. ⁵Cattedra Unesco "Educazione alla salute e allo sviluppo sostenibile", Università "Federico II" di Napoli, Naples, Italy. A full list of author and their affiliation appear at the end of the paper. ✉email: giovanna.muscogiuri@unina.it

Table 1. Association with cancers from different sites with excess body fatness, as reported by the International Agency for Research on Cancer Handbook Working Group [5].

Level of evidence	Type of cancer	
Sufficient	Esophagus: adenocarcinoma	Corpus uteri
	Gastric cardia	Ovary
	Colon/rectum	Kidney, renal cell
	Liver	Meningioma
	Gallbladder	Thyroid
	Pancreas	Multiple myeloma
	Breast, postmenopausal	
Limited	Male breast cancer	Diffuse large B-cell lymphoma
	Fatal prostate cancer	
Inadequate	Esophagus: squamous-cell carcinoma	Skin: cutaneous melanoma
	Gastric noncardia	Testis
	Extrahepatic biliary tract	Urinary bladder
	Lung	Brain or spinal cord: glioma

anti-inflammatory, antiproliferative, and antiangiogenic through activation of AMP-kinase [10].

The notion of inflammation as a tumor-promoting event involves an intricate grid of relations between innate resistance and acquired immunity. Obesity can impair innate and adaptive immunity with an increase of neutrophils, Th1 cells, CD8 cytotoxic T cells, natural killer (NK) cells and a decrease of regulatory T cells [11]. There is also an involvement of inflammasomes, i.e. cytoplasmic multi-protein complexes consisting of caspases able to activate several pro-inflammatory interleukins in response to endogenous or exogenous pathogen- or damage-associated molecular pattern. Inflammasome activation has been shown to be associated with insulin resistance [12].

Insulin resistance plays a key role in low-grade inflammation related to obesity and carcinogenesis. Chronic calorie excess promotes tissue desensitization to insulin with consequent insulin resistance. To achieve peripheral insulin actions, the pancreas responds with compensatory hyperinsulinemia, which has been associated with multiple cancers, like breast, endometrial, colonic, liver, esophageal, kidney, and pancreatic cancers [5]. The mitogenic effects of insulin signaling involve insulin receptor A and the proliferative p3K/AKT/mTOR pathways [13]. Moreover, insulin can activate several downstream components that are instrumental for insulin-like growth factors (IGFs)-dependent mitogenic actions. Hyperinsulinemia increases liver production of IGF-I and, through downregulation of IGFs carriers, enhances local IGF-I bioavailability [14]. Stimulation of IGF-I receptor and hybrid insulin/IGFs receptors can elicit mitogenic, proliferative, and antiapoptotic actions in peripheral tissues.

Hormonal consequences of hyperinsulinemia encompass blunted liver production of sex hormone-binding globulin, which allows for increased local bioavailability of free sex hormone, together with increased androgen production from ovaries and adrenals [15]. In parallel, the aromatase activity of AT enhances estrogen production and further increases local bioavailability of unbound estrogens, with consequent increased risk of carcinogenic effects of estrogens on estrogen-sensitive tissues. With regards to breast cancer, although obesity is inversely associated with progesterone receptor (PR)- and ER-positive premenopausal breast cancer, it is a recognized risk factor for postmenopausal

hormone receptor-positive breast cancer. Hence, the risk of breast cancer is greater in postmenopausal women with obesity, especially for BMIs $>35.0 \text{ kg/m}^2$ [16]. A significant association has been highlighted between breast cancer risk and insulin and IGF-I levels. Moreover, breast cancer risk is associated with leptin levels, and mice with deficient peripheral leptin receptor exhibited a decreased mammary tumor growth and progression as compared to wild-type mice [17], while an increased tumor incidence and invasiveness were found in mammary tissues of mice with diet-induced obesity through transcriptional programming regulated by leptin [18]. In vitro studies further supported a role for leptin in cell proliferation, migration, angiogenesis of breast cancer stem cells, involving in a complex signaling network with Notch and IL-1 [19].

Evidence of a relationship between obesity and certain cancers of the digestive tract emerged in experimental and epidemiological studies. Insulin elicits a proliferative, antiapoptotic, and pro-inflammatory effects on cell lines, tumor tissue, and animal models of colorectal cancer (CRC) [20]. In CRC, the anticarcinogenic effects of adiponectin [21], as well as the promitogenic effects of TNF- α , IL-6, IL-13, and IL-1 β have also been documented [22, 23]. At the epidemiological level, an obese BMI is exposed to an increased risk of early-onset CRC, with a significant age \times BMI interaction observed for ages <50 years in both sexes [24]. With regards to hepatocellular carcinoma (HCC), its relation to obesity is essentially mediated by metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) [25]. Insulin resistance amplifies de novo lipogenesis and increases the flux of FFAs, which leads to mitochondrial dysfunction with consequent oxidative stress, endoplasmic reticulum (ER) stress, and activation of unfolded protein response, all of which contribute to liver inflammation. Important contributors of increased HCC risk in obesity are represented by dysregulated adipokine secretion and amplified expression of signaling pathways associated with hepatocarcinogenesis, such as nuclear factor erythroid 2 related factor 1 (Nrf-1), NF- κ B, mammalian target of rapamycin (mTOR), PI3K/phosphatase and tensin homolog (PTEN)/Akt, and Janus kinase/signal transducer and activator of transcription (JAK/STAT) [26]. Epidemiological studies and meta-analyses showed a 17% and 89% higher risk of HCC in the presence of overweight and obesity [27], respectively, and pinpointed a 25% increased risk of HCC for each 5 kg/m^2 increase in BMI [28]. Finally, obesity and gastroesophageal reflux disease (GERD) are main risk factors for oesophageal adenocarcinoma (EAC). In epidemiological studies obesity is associated with EAC [28]. The link is particularly robust for abdominal obesity, Barrett's oesophagus and EAC, and persists after adjusting for GERD [29]. In EAC, the IGF pathway seems to play a more dominant role than insulin, and associations have also been found between leptin/adiponectin, Barrett's oesophagus and progression to EAC [30].

INTESTINAL MICROBIOTA

Microbiota encompass $\sim 10^{14}$ microorganisms composed by bacteria, eukaryotes, viruses, and archaea positioned in body's interfaces with the environment, i.e. skin, respiratory tract, gastrointestinal system, and urogenital apparatus [31]. Human microbiome is associated with systemic health in relation to substrate metabolism, energy balance, nervous and cardiovascular functions, immunity and inflammation, and it is influenced by early life events, feeding and dietary habits, growth processes, life-style, metabolic external and internal stressors factors, aging and diseases [32]. Gut microbiota contribute to absorption of dietary fats and fat-soluble vitamins, digestion of carbohydrates and polysaccharides, bile acid-related metabolism and fermentation of nondigestible food components that are ultimately metabolized in components capable of intercellular signaling such as short-chain fatty acids (SCFAs), trimethylamine-N-oxide,

bile acids, incretin hormones, polyamines, polyphenols and vitamins [33]. In the gut, microbiota preserve the integrity and permeability of the intestinal epithelial barrier through tight junction proteins, a normal endocannabinoid system tone and lipopolysaccharide (LPS) detoxification by intestinal alkaline phosphatase [34]. LPS is a microorganism-derived pro-inflammatory component released in the colon upon death of gram-negative bacteria. High-fat diet is particularly effective in altering microbiota composition and favouring LPS absorption across the intestinal barrier through chylomicrons, thereby stimulating a pro-inflammatory condition [35].

With regards to energy states, gut microbiota is addressed as one of the leading factors accompanying and pathogenetically contributing to obesity and its metabolic associates, e.g. diabetes and dyslipidaemia, NAFLD, atherosclerosis and cardiovascular diseases, as well as kidney disorders [36]. Causal mechanisms involve regulation of energy extraction from nutrients, the ability of microorganisms to ferment undigested dietary polysaccharides generating SCFAs with their lipogenic actions, the promotion of transcription factor-1 binding expression, and a decreased liver fatty acid oxidation [36]. In animal and human studies, main changes in gut microbial populations associated with obesity and metabolic diseases involve a low diversity in phyla and a rise in the *Firmicutes:Bacteroidetes* ratio, which are partly reverted upon dietary/caloric manipulation [37]. The severity of obesity has an additional detrimental impact on gut microbiota, especially in terms of microbial gene richness, which is poorly improved after weight loss due to bariatric surgery, regardless of metabolic improvements [36]. Additional changes in gut microbiota composition have been related to age and gender. In elderly subjects, a lower number of anaerobic bacteria, including *Bifidobacteria*, and a higher number of enterobacteria, have been documented [38]. Age-related modifications of intestinal microbiota recognize multiple causes, such as tissue aging, pathophysiological abnormalities, alterations in taste and smell perception, gastritis and achlorhydria, and dietary modifications. Gender-specific differences in gut microbiota have also been documented, particularly in the *Bacteroides-Prevotella* microbial group, which are higher in males than females [39]. These gender differences are likely explained by actions of sex-related hormones on environment-microbiota interaction [40], as shown in experimental gonadectomy and subsequent sex steroid restoration [40, 41]. Noticeably, richness and diversity of gut microbiota affects systemic estrogens through activities of beta-glucuronidase and beta-glucosidase enzymes, which are responsible of estrogen deconjugation and conjugation [42]. A switch in the enzymatic activities associated with the estrobolome, i.e. the entire genetic set of bacteria that process estrogens, can have an impact on the development of estrogen-dependent cancers.

MICROBIOTA AND CANCER

Microbiota can influence the risk of cancer both directly, through dysbiotic actions in the developing cancer site, or indirectly, through predisposition to metabolic disorders and influence on sex hormone status [43]. In obesity, chronic pro-inflammatory state is linked to dysfunctional gut barrier and LPS leakage, which leads to metabolic endotoxaemia [33–35]. Subsequent exposure of peripheral tissues to immunogenic bacterial components can promote a pro-inflammatory status linked to the promotion of self-renewing tumor growth factors. Obesity, insulin resistance and unhealthy dietary factors can, therefore, act to impair commensal microbiota homeostasis, promote the release of growth factors, alter the immune surveillance, and stimulate cancer cell proliferation and invasion [44, 45]. The microbiota is separated from the host's epithelial cells, which is important for regulating immune activity and supporting the host-microbe association. The mucus produced by the intestinal cells of the

calyx, together with antimicrobial peptides produced by the Paneth cells, limit the interaction between the microbiota and the host's immune system. Altering the intestinal barrier and functional biofilms that protect against pathogens invasion can contribute to enhancing the inflammation-mediated proliferative stimulus, hence creating a fitness interdependence between harmful microbes and cancer cells [46]. While cancer is considered a disease mainly caused by environmental and genetic factors, microorganisms are implicated in up to 20% of cases of human neoplasms [47]. Microbes can become part of the tumor microenvironment by influencing the growth and spread of cancer and several examples of an association between bacteria and carcinogenesis exist to date. A such, *Helicobacter pylori* (*H. pylori*) has been associated with noncardiac gastric cancer and lymphoma [48], and its virulence is reportedly connected with the A gene (CagA) associated with cytotoxin and the secretion of virulence factors to promote chronic inflammation, oxidative stress and host DNA damage [49]. Also relevant is *Fusobacterium nucleatum*, a member of oral microbiota, which expresses FadA that is capable of modulating the host's E-cadherin and activating β -catenin, thus amplifying expression of transcription factors, oncogenes, Wnt genes and inflammatory genes, associated with CRC [50]. Another bacterial species associated with the carcinogenic process is *Escherichia Coli* (*E. Coli*), that expresses the pathogenicity island pks coding for the genotoxin colibactin, which alters p53 expression [51]. Further, *Bacteroides fragilis* produces the BFT toxin which activates the Wnt/ β -catenin pathway and the nuclear- κ B factor promoting cell proliferation, inducing the production of inflammatory mediators thus inducing carcinogenesis [52]. Moreover, the microbiota can induce carcinogens through metabolites that are capable of modulating inflammation, carcinogenesis, immune response, and DNA damage. Further, pancreatic cancer has been associated with oral dysbiosis mediated by *Firmicutes*, *Proteobacteria*, bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group, and *Actinobacteria* [53]. Periodontitis has also been related to lung carcinogenesis [54].

A schematic representation of the links between obesity, microbiota, and cancer is presented in Fig. 1.

Among the obesity-associated cancers, a direct or indirect involvement for microbiota has been identified for several cancers that are followingly summarized.

Breast cancer

The human breast is not a sterile tissue, as it contains microorganisms organized in a specific microbiome niche which is distinct from that of overlying skin tissue [55]. This microbiome works to preserve healthy breast status both directly by inactivating harmful metabolic substances and indirectly via stimulation of resident immune response [56]. Although the bacterial composition of breast does not seem to vary between tumor tissue and adjacent normal tissue, both at the population and individual level [57], variations in abundance of *E. Coli* and other bacterial profiles have been documented between healthy and breast cancer women [57]. A discriminatory role for *Methylobacterium* abundance between normal and adjacent breast tumor tissue has also been reported, suggesting a potential local contribution of this bacterium in breast cancer development [58]. Gut dysbiosis associated with metabolic disorders has been hypothesized to influence the risk of postmenopausal breast cancer by altering enteric regulation of estrogen metabolism modulated by deconjugation reactions [59]. In this context, dietary components, such as fats, play a relevant role in promoting the overgrowth of *Proteobacteria* species, through the synthesis and excretion of bile acids, which can be catabolized by gut commensal bacteria into metabolites. Beta-glucuronidases synthesized by these organisms can deconjugate estrogens and increase circulating estrogens thereby contributing to modifications in the

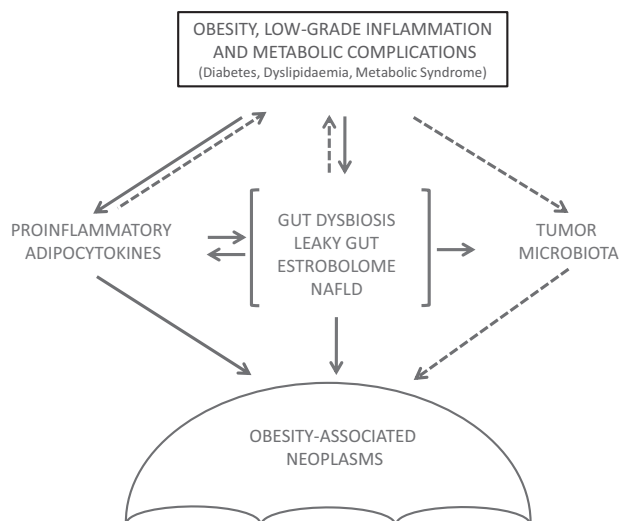


Fig. 1 A schematic representation of the association between obesity, microbiota, and cancer. NAFLD nonalcoholic fatty liver disease. Estrobolome: the aggregate of the enteric bacterial genes whose products are capable of metabolizing estrogens.

estrobolome [60]. Alternatively, the phytoestrogen enterolactone, which interacts with the gut microbiome and can be converted into enterolactone glucuronide has been recently associated with a protective role on breast cancer in postmenopausal women [61].

Colorectal cancer

A role for microorganisms in CRC development has long been known. *Escherichia*, *Enterococcus*, *Bacteroides*, and *Clostridium* genera influence the induction of aberrant crypt foci by 1,2-dimethylhydrazine in rats [62]. In humans, CRC-associated microbiota is different compared with healthy gut microbiota, in terms of species richness, abundance of protective species such as *Roseburia*, and increases in procarcinogenic species such as *Bacteroides*, *Escherichia*, *Fusobacterium*, and *Porphyromonas* [63]. CRC is associated with dysbiosis and a meta-analysis of metagenomic studies highlighted a direct association between CRC and seven enriched bacteria with enterotoxigenic activities encompassing, among the others, *B. fragilis* and *Prevotella intermedia*, as well as negative correlations with probiotic bacteria such as the butyrate-producer *Clostridium butyricum* and the lactic acid bacterium *Streptococcus thermophilus* [64]. Substantial changes in abundance of specific bacteria can be detected in patients with CRC and might serve as biomarkers for disease screening, prognostication and prediction of treatment response [65].

Hepatocellular carcinoma

In the liver, not containing a known microbiota, HCC has been linked to intestinal bacteria through metabolites [66] such as Pattern Recognition Receptors ligands, which recognize pathogen-associated molecular patterns (PAMPs), the latter involved in activating innate immune responses and protecting the host from infection. LPS, which belongs to a prototypical class of PAMPs, is capable of promoting liver carcinogenesis when bacteria cross the intestinal barrier and enter the systemic circulation [67]. Deoxycholic acid, a secondary bile acid, promotes the secretion of inflammatory cytokines in hepatic stellate cells, supporting the development of HCC [68]. Moreover, secondary bile acids, through the signaling of β -catenin, kinases 1 and 2 (ERK 1/2) regulated by the signaling of the activator of protein 1 (AP1), stimulate the invasiveness and proliferation of colon cancer cells [69]. They also damage the architecture and function of the colon

epithelium through multiple oxidative DNA damage, inflammation and activation of the NF- κ B pathway [70].

Prostate cancer

In healthy conditions, the prostate contains only a few microbial organisms, which remain indolent if the luminal epithelial barrier and antimicrobial prostate secretions are normal. Prostate disorders, impaired antimicrobial activity and loss of epithelial barrier integrity can promote the overgrowth of pathogenic urinary microbiome in the prostate ducts [71], enabling microorganisms to penetrate through the epithelial barrier and reach the stromal prostate component, where they promote local inflammation [71]. In chronic prostatitis/chronic pelvic pain syndrome, the urinary microbiome shows a higher representation of *Clostridia* and *Bacteroides* and a higher alpha-diversity, as compared to controls with higher prevalence of Bacilli bacteria such as *Lactobacillus* and *Staphylococcus* [72]. Similar alterations occur in seminal fluids of men with prostatitis, confirming the pro-inflammatory role of male urinary dysbiosis. While men with and without biopsy-proven prostate cancer showed no variation in the load and diversity of urinary microbiome, a greater representation of pro-inflammatory and pro-carcinogenic bacteria, such as *Streptococcus anginosus*, *Anaerococcus lactolyticus*, *A. obsiensis*, *Varibaculum cambriense* and *Propionimicrobium lymphophilum*, was noticed in a subset of patients with prostate cancer in relation to cancer grading and coexistent prostate inflammation [73]. This pro-inflammatory microenvironment could facilitate the risk of chronic prostatitis and predispose to the occurrence and progression of prostate cancer [74]. Less clear is the involvement for gut dysbiosis in the risk of prostate cancer of persons with obesity and metabolic impairment. Alterations in sex steroids (e.g., decreased testosterone, elevated oestrogen levels), oxidized LDL cholesterol and low-grade inflammation are related to the progression of BPH and chronic prostate inflammation, and could promote a pro-inflammatory microenvironment with a higher risk of prostate cancer development [75].

MICROBIOTA AND ANTINEOPLASTIC TREATMENTS Chemotherapy

Studies have focused on the ability of gut microbiota on influencing the course and/or outcome of different antineoplastic therapies, such as chemotherapy, radiotherapy, and immunotherapy. Several antitumor compounds have been studied in relation to the metabolic modifications generated by gut microbiota. However, only a few have been found to be directly metabolized by gut microbiota to date, such as the antimetabolite methotrexate, the radiation-sensitizer misonidazole, and the topoisomerase-I inhibitor irinotecan. This latter represents a parenteral anticancer therapy that is used for CRC. Its metabolism first consists of liver metabolism and subsequent biliary excretion into the gastrointestinal tract, where beta-glucuronidases of microbial organisms, particularly of *Firmicutes* phylum, exert a conversion into the active metabolite SN38, which is responsible for local mucosal toxicity associated with diarrhea [76]. This mechanism has been exploited to investigate the potential effect of antibiotics or specific glucuronidase inhibitors in animals, as well as of probiotics in humans to control irinotecan-induced diarrhea and gut toxicity, with partial benefits [77]. In addition to the actions of gut microbiota on anticancer drugs metabolism and absorption, an indirect influence of gut microbiota involves modification of cytochrome P450 gene expression that is associated with a faster metabolism of oral xenobiotics, suggesting an indirect role of gut microbiome in the modulation antineoplastic drug responses and susceptibility to adverse events [78]. A modulatory effect of gut microbiota has also been identified for gemcitabine through the action of *E. Coli* even if the underlying mechanisms remain unclear. Conversely, a synergic effect of gut microbiota with

platinum-derived anticancer drugs, such as cisplatin and oxaliplatin, has been demonstrated on their ability to inhibit DNA replication and intra-strand platinum–DNA adducts, thereby preventing formation of DNA double-strand breaks [79]. In particular, the underlying mechanism could be explained by the evidence that gut microbiota-depleted mouse models have shown a decreasing of ROS-mediated effect of platinum-derived compounds on tumor cells, due to an altered activation of myeloid differentiation primary response 88-associated innate immune response [79]. Experimental evidence also exists that the response to the alkylating agent cyclophosphamide interacts with the gram-negative species *Barnesiella intestinihominis* (*B. intestinihominis*) to modulate infiltration of interferon- γ producing T cells [80]. This agent selectively impacts the diversity of gut microbiota and increases gram-positive bacteria such as *Enterococcus hirae* (*E. hirae*), which translocate into mesenteric lymph nodes and induce a significant increase of the intra-tumoral CD8 + T cell/T regulatory cell ratio as well as T-helper (TH) 17 and memory TH1 lymphocytes, thereby promoting an antineoplastic adaptive immune response in the spleen [80]. By reverse, synergistic actions of cyclophosphamide and gut microbiota are confirmed in mice depleted of Gram-positive bacteria [81]. An immune response related to the presence of *B. intestinihominis* and *E. hirae* has been associated with a more favorable prognosis in patients with advanced lung or ovarian cancer treated with chemo-immunotherapy [82].

Antineoplastic immunotherapy

Anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA4) immunotherapy induces a mucosal damage in the gut by compromising barrier integrity and facilitating the access of bacteria to the lamina propria, with activation of local innate immune response and subsequent systemic inflammation by translocated bacteria [83]. In particular, the antineoplastic immune response induced by anti-CTLA4 immunotherapy involves an anti-commensal effect against *Burkholderiales* and *Bacteroidales* (*Bacteroides thetaiotaomicron* and *Bacteroides fragilis*), which are responsible for gut mucosal disruption, translocation of bacterial species, and adjuvant support for antitumour immune response [84]. The relevance of this mechanism is supported by clinical evidence that patients with metastatic melanoma treated with ipilimumab exhibit greater survival and progression-free survival in relation to abundance of *Fecalibacterium* and other *Firmicutes* as compared to microbiota enriched in *Bacteroides* [85]. In melanoma patients, an association has also been identified between gut microbiota profile, particularly abundance of the *Ruminococcaceae* family, and response to treatment with anti-programmed cell death protein-1 (anti-PD1) antibodies [86]. This effect is corroborated in experimental melanoma models, where abundance and/or treatment with *Bifidobacterium* have been associated with anti-PD1 antitumor activity, supporting the possibility that this bacterial species represent an interesting adjuvant therapy to checkpoint inhibitors such as anti-PD1 drugs [87]. On the other side, antibiotic treatments have been found to significantly reduce the effectiveness of anti-PD1 immunotherapy in patients with advanced colorectal carcinoma and non-small cell lung cancer in terms of primary progressive disease, shorter overall survival, and progression-free survival [88].

MODIFICATION OF MICROBIOTA THROUGH WEIGHT LOSS AND CANCER RISK

Randomized controlled trials and intervention studies suggested that intentional weight loss, either through dietary modification and/or physical activity, are beneficial for cancer risk. A 3-year follow-up in a large female cohort found significant reductions in the risk of obesity-related cancer in women who intentionally lost more than 5% of their body weight [89]. In a meta-analysis on

mortality risk among breast and CRC survivors, an inverse association with physical activity before cancer diagnosis was reported [90]. Conversely, observational studies showed that weight gain after breast cancer diagnosis was inversely associated with disease-free survival [91].

Bariatric surgery, the most effective treatment for sustainable weight loss in patients with obesity, elicits short and long-term weight-reducing effects due both to caloric restriction and modification of metabolic regulators acting at the gastrointestinal and central level [92]. Bariatric surgery has exhibited the ability to reduce the risk of obesity-related mortality and morbidities when compared with intensive medical and lifestyle interventions [93], as well as it is associated with an overall reduced incidence in cancer from different sites [94]. However, a site-specific cancer preventive effect of bariatric surgery seems to exist. For example, growing interest has focused on CRC risk after bariatric surgery and, while a systematic review and meta-analysis suggested a beneficial effect of bariatric surgery on CRC risk [95], a prospective study showed a time-dependent increase in CRC risk 10 years or more after bariatric surgery [96]. In line with this finding, a large case-control study using a propensity score-matching methodology found an elevated risk of CRC in people with obesity subjected to bariatric surgery [97]. In the case of hormone-related cancers, bariatric surgery may act favorable on cancer prevention. Particularly, a study in women with BMI > 35 kg/m² found that bariatric surgery reduced the risk both of premenopausal and postmenopausal breast cancer, with an effect that was stronger among ER– premenopausal cases and ER+ postmenopausal cases [98], although data analysis robustness of this study has been questioned. To explain the previously reported discrepancies, it has been hypothesized that persistent gut dysbiosis could play a role. Bariatric surgery induces changes in gut microbiota diversity, with relative increases in *Bacteroidetes*, *Proteobacteria*, and *Gammaproteobacteria*, and a decrease in the abundance of *Firmicutes* [99, 100]. These functional and taxonomic changes could originate from postsurgical gastrointestinal rearrangements, as well as changes in eating habits and macronutrients consumption. Whether these changes promote a shift towards a lean microbiome phenotype is, however, uncertain. In fact, a study on changes of gut microbiota in obese persons subjected to bariatric surgery revealed an improved but still impaired postsurgical microbial diversity, which is associated with the risk of CRC [101]. Noticeably, postsurgical dietary modifications could even favor the production of harmful metabolites and increase the risk of DNA damage, leaky gut and inflammation, as exemplified by a study on gastric bypass showing an increase in bile acids, which can induce cytotoxic effects and the proliferation of malignant cells [102]. Hence, persistent intestinal dysbiosis, ongoing excess body weight after bariatric surgery, postsurgical modifications in diet composition and digestion, and exposure to proliferative agents like bile acid could play a role on carcinogenesis that awaits further clarification.

MICROBIOTA MANIPULATION AND CANCER

Prebiotics

Manipulation of intestinal microbiota through prebiotics has beneficial effects on their abundance and diversity, microbial production of SCFA, metabolic dysfunction, WAT accumulation, and immune functions [103]. Prebiotics are nutrients not easily digested by humans, which are capable of stimulating the growth and/or activity of beneficial bacterial species in the gut. Examples of prebiotics include fructans, inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose. Inulin modulates inflammation and metabolic endotoxemia, causes a relative intestinal increase in *Bifidobacterium* and *Faecalibacterium prausnitzii*, is negatively correlated with serum LPS and, through effects on gastrointestinal hormones like glucagon-like peptide-1 (GLP-1),

peptide YY (PYY), and ghrelin, can influence food intake, glucose homeostasis and energy balance [104]. It has also been shown that oligofructose, like inulin, elicits adjuvant effects on cytotoxic treatment in cancer-bearing mice [105]. Interestingly, metformin has been found to promote the growth of *Caenorhabditis elegans* (*C. Elegans*), inhibiting the metabolism of methionine, and influencing the metabolism of folate [106]. Collectively, prebiotics show promising effects in preventing carcinogenesis by promoting growth and/or activity of health-promoting microorganisms.

Probiotics

Living microorganisms in a state of cryptobiosis confer health advantages for the host if administered in adequate quantities [107]. In experimental conditions, several *Lactobacilli* strains have shown the ability to attenuate diet-induced weight gain, improve insulin sensitivity and glucose homeostasis, reduce liver steatosis, and decrease pro-inflammatory macrophage infiltration in WAT and liver. In humans, bacterial strains like *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Akkermansia muciniphila* (*A. muciniphila*), have displayed anti-obesity effects [108]. Immunomodulatory and anticancer activities of probiotics such as *Lactobacilli* (*Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* GG) involve activation of NK cells or maturation of dendritic cells (DC) [109], or induction of ferrichrome, which promotes cancer cell apoptosis through JNK signaling pathway ([110]. In mice with HCC, a mixture of *L. rhamnosus* GG, *E. Coli* Nissle 1917 and VSL#3 shifted the gut microbial community toward beneficial bacteria like *Prevotella* and *Oscillibacter*, and exerted antiangiogenic, anti-inflammatory and antiproliferative effects [111]. Likewise, transfer of *Bifidobacterium* short or *Bifidobacterium longum* in mice lacking *Bifidobacteriales* has been shown to reduce melanoma growth and restore anti-melanoma CTL responses [87].

Fecal transplant

Fecal microbiota transplantation technique (FMT) involves the exchange of the intestinal microbiota between individuals and has been used to treat bacterial infections, particularly from *Clostridium difficile*. FMT is showing promising results for the treatment of obesity, metabolic syndrome and insulin resistance [112]. In terms of microbial composition, FMT recipients develop a relative abundance of *Ruminococcus bromii* and *Roseburia intestinalis*, or *A. muciniphila* species, all of which are associated with improvements in insulin sensitivity. However, while the movement of specific microbes improves insulin sensitivity, the nature of the microbiome manipulated by FMT appears to be unstable. Notwithstanding the advantage conferred by FMT on dysglycaemia in short-term studies, i.e. lasting <6 weeks, effects reportedly vanish in long-term studies [113]. This could be due to the fact that obesity is associated with chronic low-grade inflammation, which in itself causes intestinal dysbiosis [114]. The rationale of FMT for cancer management includes reconstruction of intestinal microbiota, amelioration of bile acid metabolism, and modulation of immunotherapy efficacy [115]. In mice, FMT could alleviate the gut dysbiosis caused by the microbiota, and restore the eubiosis, thus reducing inflammation and proliferative and carcinogenic pathways [43]. Preclinical evidence of an advantageous cancer-related utilization of FMT involves CRC, pancreatic cancer, HCC, breast cancer, and melanoma [115]. Whether FMT can reduce carcinogenesis and tumor progression in humans remains a potential area of investigation.

Ketogenic diet

Excessive consumption of refined sugars elicits harmful effects on human health in terms of susceptibility to obesity, microbial diversity, and proliferation of pathogenic species, such as *C. difficile* and *C. perfringens* [116]. Moreover, refined sugars are an energy source for cancer cells [117]. In recent years, the low-calorie ketogenic diet (KD) approach is becoming increasingly

popular for the treatment of cardiometabolic disorders and cancer. Low-calorie KD provides <800 kcal/day and a carbohydrate amount <30 g/day, consisting of about 13% of the total energy, while fats and proteins account for 44% and 43%, respectively [118]. KD is capable of producing physiological ketosis, i.e. an increase in ketone bodies, acetoacetate, and β -hydroxybutyrate [118]. Ketone bodies act as energy substrates, control mitochondrial metabolism and energy, have favorable effects on microbiota. KD show positive metabolic effects in obesity, in terms of oxidative stress, ROS/superoxide production, lipid peroxidation, protein oxidation, inflammation and immune intestinal cell function, and microbiota diversity. Recently, KD has proven able to decrease growth of cancer in animal models of malignant glioma, prostate cancer, CRC and gastrointestinal cancer, as well as in humans with brain or prostate cancer, although the inhomogeneous design of available studies warrants caution [119]. When used as an adjuvant therapy, KD is capable of sensitizing cancer cells to chemotherapy and radiotherapy treatments [120]. Microbiota modification induced by KD is thought to play a key role in some cancers and the crosstalk between different organs also via tumor suppressor metabolites. Like microbiota, KD is responsible for the production of SCFA, which can aid cancer treatment and cancer prevention. Therefore, KD and KD-induced microbiota could synergistically contribute to prevent tumorigenesis and constitute promising strategies to slow down carcinogenesis and increase the effectiveness of cancer therapies.

CONCLUSIONS

The symbiotic relationship between the microbial community and the host is not just an innocent bystander in metabolic alterations predisposing to cancer development. Dysbiosis is, both locally and systemically, an integral part of carcinogenesis that intervenes to modulate responsiveness and tolerance of antineoplastic therapies, particularly immunotherapy. Influencing the microbial community could aid the therapeutic approach to cancer management, in relation to reorganization of intestinal microbiota, improvement of harmful metabolites, antitumoral immune response, and modulation of anticancer therapy. A crucial step towards implementation of anticancer strategies is represented by exogenous approaches capable of modulating gut microbiota to improve intestinal dysbiosis and, potentially, influence cancer outcomes in different sites. Nevertheless, there is a critical need to translate emerging experimental evidence into robust clinical results, and to support the attained clinical findings with randomized controlled trial outcomes. This strategic course would not only help to recollect and consolidate current evidence to legitimate the validity of studies presented, but could also represent a utility prediction tool for microbiota manipulation in adjuvant therapeutic management of neoplastic disorders.

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Lubrano, Paolo Emidio Macchia, Silvia Magno, Paolo Marzullo, Davide Menafrà, Silvia Migliaccio, Fabrizio Muratori, Giovanna Muscogiuri, Raffaele Napoli, Caterina Pelosini, Francesca Pivari, Rosario Pivonello, Eleonora Poggiogalle, Gabriella Pugliese, Gabriele Riccardi, Alberto Ritieni, Fiammetta Romano, Domenico Salvatore, Alessandro Sanduzzi, Ferruccio Santini, Silvia Savastano, Paolo Sbraccia, Giovanni Scambia, Laura Soldati, Giovanni Spera, Maria Grazia Tarsitano, Dario Tuccinardi, Olga Vaccaro, Mary Venneri, Samir Sukkar, Roberto Vettor.

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to G.M.

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ON BEHALF OF THE OBESITY PROGRAMS OF NUTRITION, EDUCATION, RESEARCH AND ASSESSMENT (OPERA) GROUP

Annamaria Colao⁵, Silvia Savastano⁵, Silvia Magno⁶, Andrea Di Nisio³, Fiammetta Romano⁵, Giovanna Muscogiuri⁵, Eleonora Poggiogalle⁷, Mary Venneri⁷, Alessia Liccardi⁵, Maria Grazia Tarsitano⁷, Luigi Barrea⁵, Laura Di Renzo⁷, Dario Tuccinardi⁷, Massimiliano Caprio⁷, Maria Angela Guzzardi⁶, Caterina Pelosini⁶, Gabriella Pugliese⁵, Filomena Bottiglieri⁵, Paolo Marzullo Sara Aprano⁵, Silvia Bettini Davide Menafrà⁵, Gianluca Gortan Capellari⁸, Daniela Laudisio⁵, Francesca Pivari⁹, Caterina Brasacchio⁹, Andrea Lenzi⁷, Fabrizio Muratori¹⁰, Ferruccio Santini⁶, Luca Busetto³, Paolo Sbraccia⁷, Laura Soldati⁹, Domenico Salvatore⁵, Carolina Di Somma⁵, Laura Di Renzo Dario Giugliano⁵, Lucio Gnessi⁷, Brunella Capaldo⁵, Gabriele Riccardi⁵, Rocco Barazzoni⁸, Brunella Guida⁵, Maurizio Bifulco⁵, Katherine Esposito⁵, Roberto Vettor³, Paolo Emidio Macchia⁵, Andrea Di Nisio Felipe Casanueva¹¹, Carla Lubrano⁷, Francesco Beguinot⁵, Giovanni Spera⁷, Annamaria Belfiore⁵, Luigi Di Luigi⁷, Laura Soldati Alberto Ritieni⁵, Raffaele Napoli⁵, Olga Vaccaro⁵, Samir Sukkar¹², Carlo Alvirgi⁵, Rosario Pivonello⁵, Giuseppe Bellastella⁵, Giovanni Scambia⁷ and Giuseppe Bifulco⁵

⁶Pisa, Italy. ⁷Roma, Italy. ⁸Trieste, Italy. ⁹Milano, Italy. ¹⁰Como, Italy. ¹¹Spagna, Italy. ¹²Genova, Italy.