Review Article

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Role of microbial dysbiosis in carcinogenesis & cancer therapies

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The human body supports a heterogeneous population of microorganisms. Every microorganism has the ability to contribute to the unique microenvironment around it. The aim of this review is to discuss the changes in the microbial population and their relative abundance across different ecosystems of the human body, the interactions within the microbial communities, metabolites they secrete to their external environment, their immunomodulatory functions, their signal transduction pathways and how these respond to environmental stimuli such as various diets, alcohol and drug consumption, smoking and finally suggest new therapeutic approaches. The microbiota may leads to cancer through inflammation mediated mechanisms which modulate immune responses, or produce carcinogenic metabolites and genotoxins, or deregulate cell proliferative signalling pathways. The identification of these molecular mechanisms in carcinogenesis may lead to better treatment strategies. In this review we have tried to explore the changes in microbial composition between cancer and normal tissues and what molecular mechanisms provide a connecting link between microbial dysbiosis and cancer.

Key words Cancer therapies - carcinogenesis - dysbiosis - immunomodulation - microbiome

Microbiome plays an important role in maintaining the normal physiology of the human body. The changes occurring in the microbial composition induce production of toxins, chronic inflammations and carcinogenic metabolites through several mechanisms. Dysbiosis can be defined as an imbalance in number and types of microbial population, which can modulate microenvironment and homeostasis in host through over proliferation of a certain microbial species. Thus, dysbiosis may directly or indirectly contribute to carcinogenesis in human beings[1]. Studies on microbiome are mainly concentrated on regions such as hair[2], mouth[3], nostrils[4], gut[5], stomach[6] and colon[7] of the human body.

The conventional culture-based microbial studies are inadequate to understand species diversity and relative abundance. In the conventional microbial culture based technique, *16s rRNA* gene sequencing is a PCR based technique, which provides a method for the identification of bacterial member in microbiome. The mechanism involves amplification of prokaryotic small ribosomal RNA (16s rRNA) gene by PCR[8,9]. The primer used in PCR binds to a conserved region of rRNA, while the extension step occurs on a highly variable region of rRNA, which is specific for each species. To overcome limitations, emerging molecular techniques for the microbiome research including next-generation sequencing, advanced culture

technologies and its combination with metabolomics are also used in research[10].

Role of dysbiosis of skin microbiome in carcinogenesis

Human skin microbiome contains a highly diverse community of hundreds of species inhabiting the skin[11]. Each area of skin provides a different environment with varying, temperature, moisture, *p*H, salinity, sebum content and intrinsic factors. These differences along with other lifestyle related factors determine the composition of microbiota on different skin habitats. Skin contains highly uneven surface having many invaginations with hairs and follicles, sebaceous glands; these protruding structures along with the presence of sweat glands make skin a diverse habitat and consequently, the microbes present on the skin are different in each part of the body[12]. The microbial diversity is more abundant in moist body sites, where the sweat gland produce unfavourable growth condition for the microorganisms and thereby allow the colonization of only certain microorganisms. Sebaceous glands secrete hydrophobic lipid rich sebum which acts as an antimicrobial agent in hairy areas of the skin[13,14] whereas, *Staphylococcus epidermidis* survive in commensal relationship with the skin surface and forms a part of commensal microbiota in skin. It secretes a serine protease enzyme that inhibits *Staphylococcus aureus* colonization[15]. These regulatory mechanisms on skin surface prevent changes in microbial population and resist over proliferation of certain microbial strains over the other. The microbiome inside the body is involved in the activation of immune cells, especially cells that produce inflammation[16].

An *in vivo* study on skin cancer mice model demonstrated that the colonization of certain flagellated microorganisms promoted carcinogenesis through inherent signalling mechanism in the host. The microenvironments around the wound provide favourable environment for the growth of opportunistic microorganisms, especially flagellated bacteria like *Escherichia coli* and *Pseuodomonas aeruginosa*[17]. Flagellin and other microbial particles induce inflammation and activate toll-like receptor 5 (TLR-5) mediated signalling mechanisms in wound[18]. A study confirms that topical application of flagellin on the wound increases tumour growth, while antibiotic treatment and ablation of TLR-5 produce an anti-tumourogeic effect[19]. This indicates that through microbial dysbiosis human immune system can induce tumour formation. Multiple immunity-related mechanisms have been suggested to explain the link between skin microbiome and cancer[20], but a detailed profiling of microbial population is necessary to unmask the molecular mechanisms which induce the carcinogenesis[21].

Dysbiosis of oral microbiota influences carcinogenesis

Oral microbiome occupies different ecological niches in an oral cavity, and each niche contains a different composition of microbiota. It is one of the most diverse microbial communities with more than 500-700 species[22]. Each part of the oral cavity including lips, gingivae, teeth, hard palate, buccal mucosa, tongue and floor of the mouth provides an optimum environment for the proliferation of different microorganisms in a symbiotic manner. This environmental diversification contributes to the existence of a large number of species in a small ecosystem. Epidemiological studies indicate differences in the species abundance from one site to another[23]. The oral cavity itself contains several physical and chemical factors secreted by the commensal microbiota, which render protection from various microbial pathogens through immune system activation and resource competition[24]. The commensal microbiota not only provides resistance against infectious agents but also plays a vital role in the digestion, maintenance of homeostasis, signal transduction and other cellular processes[25].

In some oral cancers, an over proliferation of some commensal microbial species such as *Streptococcus anginosus* or *Fusobacterium nucleatum* has been observed. The infection of *S. anginosus* in oral mucosa induces overproduction of nitric oxide which leads to DNA damage and finally lead to carcinogenesis[26]. Similar to *S. anginosus*, *F. nucleatum* is a pro-inflammatory, anaerobic, adherent bacterial oral pathogen. Typically, *F. nucleatum* is seen in the oral cavity, but in chronic periodontitis, the balance between the host-microbiota interactions is broken[27]. Increase in the number of *F. nucleatum* represents an example of an opportunistic infection at an immunocompromised site[28]. The chronic periodontitis leads to chronic inflammation, so the infected cells have a high risk to develop into premalignant lesions and finally into tumour[29]. A study using chronic inflammation associated tumourigenesis animal model has demonstrated that *F. nucleatum* and *Porphyromonas gingivalis* modulate Interleukin 6 (IL-6) – signal transducer and activator of transcription 3 (STAT

3) axis of inflammatory signalling pathways[27]. These bacteria also help in tumour progression through atypical activation of immunocytes, which then produce DNA damage through generation of reactive chemical species[27]. In addition to IL-6 mediated immune responses, *F. nucleatum* also promotes lipopolysaccharide-induced production of inflammatory cytokines such as tumour necrosis factor α (TNF α), IL-1β and IL-12 and IL-17[30]. These immune responses result in the upregulation of inflammation induced transcription factor such as nuclear factor kappa beta (NF-κB), which promotes tumourigenesis at the site of infection[31]. Another bacterium *P. gingivalis* increases prostaglandin-endoperoxide synthase expression through cyclooxygenase-2 gene expression and thereby causes symptoms of inflammation by bringing pro-inflammatory mediators into the site of infection[32]. These microbial mechanisms can indirectly induce inflammation mediated carcinogenesis and tumour progression.

Role of dysbiosis of gut microbiota in carcinogenesis

F. nucleatum is rarely seen in healthy human gastrointestinal tract. *F. nucleatum* has the ability to induce damage to the epithelial lining of colon[30]. An *in vivo* studies with murine models has demonstrated that specific myeloid cell group penetrates into the tumour and produces pro-inflammatory signals[31,32]. Cancer promoting property of *F. nucleatum* is mediated through FadA adhesion mechanism[33]. FadA bind to E-cadherin in the host cell and activates the β-catenin signalling pathways. FadA also promotes expression of oncogenes and regulates the inflammatory responses when the *Fusobacterium* adheres to it[34]. Unlike the chronic inflammation mediated mechanism of tumourigenesis, *Fusobacterium* can induce tumour formation by reconstruction of microenvironment around the host cells and in turn infiltrates into the tumour and promotes cell proliferation. The binding and activation of the *F. nucleatum* to the FadA motif promotes the signalling mechanism that alters epithelial barrier which leads to the loss of tight junction interactions, the mucus layer integrity, epithelial cell polarity and cell to cell adhesion[35]. These alterations in the epithelial barrier induce the invasion of the *Fusobacterium* species and accelerate cell-mediated immunity against the bacterium inside the tumour[36]. The inflammatory genotoxic condition produced by the immune cells triggers DNA damage and finally promotes cell proliferation[37] (Figure). Similar to

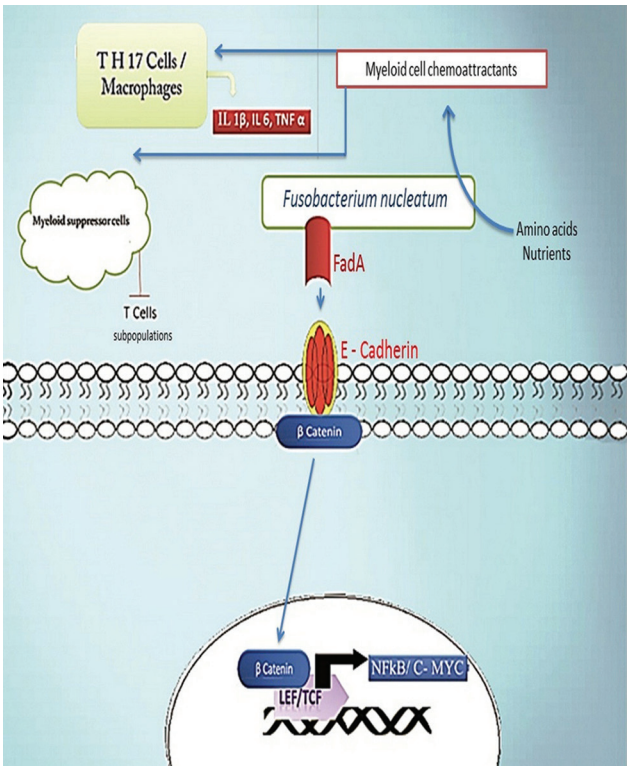

Figure. *Fusobacterium nucleatum* through FadA activates NF- kB and C-MYC transcription factors mediated cell proliferation pathway. *Fusobacterium* species produces short peptides or short chain fatty acids which act as chemo-attractants, and attracts TH 17 cells and myeloid derived suppressor cells (MDSCs) to the tumour site. TH 17 cells produce interleukin 1 beta (IL-1β), interleukin 6 (IL-6) and tumour necrosis factor α (TNF α), while myeloid suppressor cells suppress some T cell subpopulations such as CD4+ cells in tumour microenvironment· Fusobacterium also leads to the increased presence of tumour associated macrophages near tumour. Fusobacterium express FadA ligand molecules on its surface which binds to E cadherin which is an adhesion molecule in epithelial cells and activates E cadherin mediated β catenin cell proliferation pathway. Activation of β catenin signalling leads to its translocation inside the nucleus where it leads to increase in expression of oncogenes such as C-MYC and NF-kB by binding to coactivator transcription factors lymphoid enhancer factor (LEF) and T cell factor (TCF).

the β-catenin signal activation by *F. nucleatum,* the pathogenic bacteria species *Bacteroides fragilis* produces a toxin with an additional oncogenic function. This toxin cleaves β-catenin and activates signalling pathway for cell proliferation. In addition to the interactions between the immune cells and the microbial products, it will also induce DNA damage and contribute to tumourogenesis in the host. The epithelial barrier functions to separate the immune cells from this microbial microenvironment. When a tumour developes, the immune/inflammatory cells (CD4, TH1 cells, TH-17 cells, *etc*.) infiltrates the epithelial barrier and come in contact with the tumour as well as the

microbial community[38]. Activated microbial products will induce the production of IL-17 and IL-23 from myeloid cells[35]. These ILs are mediators of inflammation and promote a chronic inflammation around the tumour to form a favourable microenvironment for the growth of the tumour[39] (Figure).

Enterococcus faecalis forms a part of the intestinal microbiome. The abnormal proliferation of this bacterial strain induces DNA damage and generates colorectal carcinomas[40]. *E. faecalis* can produce extracellular superoxide (O^*_2), hydroxyl radical and H_2O_2 through demethylmenaquinone-mediated autoxidation. The ability of *E. faecalis* to induce DNA damage was proved in a mice model and it was found that these free radicals were the cause of chromosomal instability and these were associated with colorectal cancer[41]. The gastrointestinal tract develops adaptive immunity with the help of intestinal microbiota in the peripheral region. The interaction between intestinal mucosa and microbiota is mediated through Toll – like receptors, (TLRs) these interactions play a vital role in maintaining homeostasis. When the mucosal barrier ruptures, immune cells such as T cells and macrophages activate TLR-dependent pathway which increases epithelial cell proliferation and recruits inflammatory cells to the site. The TLRs may also be activated by the infectious microorganism like; *Helicobacter pylori* and *Listeria monocytogenes* through which this natural mechanism turns into a carcinogenic mechanism[42,43].

Dysbiosis of microbiome and gynaecologic cancers

As mentioned earlier, inflammatory mediated mechanisms and cytotoxic mechanisms induce tumour progression mediated by the human microbiota. The gynaecologic cancers also form a part of such cancers which develop into tumours through dysbiosis of microbiota. Studies on dysbiosis have revealed the association between change in microbial community and human papillomavirus infection[44]. A woman whose vaginal microbiota is low in *Lactobacillus gasseri* species and high in *Atopobium* possesses a healthy composition of the microbiota, while HPV-positive women show higher number of *L. gasseri* and *Gardnerella vaginalis* species[45]. The *p*H of the vaginal region supports the invasion and sustainability of the HPV virus[46]. Similarly, ovarian cancer is also influenced by the upper reproductive tract microbiota. The colonization of the pathogenic bacteria activates the inflammatory pathways in uterus and fallopian tubes which promote immunomodulation and tumourigenesis[47].

The vaginal microbiota and uterine cancer are linked by two inflammatory diseases *viz*. pelvic inflammatory disease (PID) and bacterial vaginosis. Bacterial vaginosis promotes the dysbiosis of vaginal microbiota, especially the population of *G. vaginalis, Mycoplasma hominis* and *Ureaplasma urealyticum*[48,49]. Changes in the relative population size lead to PID, as a consequence of which epithelial dysfunction and chronic inflammation occur simultaneously in the uterine region. These conditions together promote tumour growth and invasion[49].

Factors influencing dysbiosis of microbiome

Studies indicate that development of cancers is also linked to lifestyle-related risk factors. These factors form a major cause of dysbiosis, and these include smoking, diet imbalance, alcohol consumption, obesity and lack of physical exercise[50-52]. These changes can be studied through metagenomic studies, which help to identify dysbiosis and their relationship with genetic susceptibility in a comprehensive way. Diet imbalance or a specific food pattern may contribute towards cancer development via dysbiosis[53]. The risk factors such as alcohol consumption and tobacco use form a common cause of cancer when compared to other lifestyle-related factors. Although the diet related disorders account for a negligible portion of cancer cases, but some dietary habits such as high meat or pork intake, low vegetable or fruit consumption increase susceptibility to cancer[54]. Cancer preventive diet can overcome some effects caused due to changes in the microbial community. The diet with green vegetables and fruits has high level of antioxidants and carotenoids that protect cells from triplet sensitizers, singlet oxygen, and radical intermediates and maintains a normal commensal microflora inside the body [55].

Acetaldehyde is considered as teratogenic, genotoxic and mutagenic agent because it reacts with DNA and produces a N2-ethylidenedeoxyguanosine adduct[56-58]. It also interacts with deoxyguanosine in the DNA to form 1 N2-propano-2-deoxyguanosine adduct[59]. Therefore, the accumulation of acetaldehyde may lead to permanent alterations in the genetic material. Many investigations on microorganisms in the oral cavity have shown that one of the reasons for an increase in the alcohol dehydrogenase level

when compared to aldehyde dehydrogenase is due to dysbiosis in the oral microbiome[60]. *Neisseria* is the part of commensal microflora of oral cavity, some of the non-pathogenic *Neisseria* species produce extremely high amount of alcohol dehydrogenase enzyme in the oral cavity and may lead to carcinogenesis[61,62]. On the other hand, an elevated level of *Streptococcus salivarius, Candida albicans* and some Gram-positive bacteria and yeast show ability to produce acetaldehyde in the presence of alcohol in the oral cavity [63].

Another reason for the dysbiosis in the oral cavity is tobacco consumption. The immunomodulatory functions of tobacco allow invasion of pathogenic microorganisms to the oral cavity, nasal mucosa, throat, trachea and lungs[64]. In a comparative study on smokers and non-smokers, *Treponema denticola, Prevotella intermedia, F. nucleatum, P. gingivalis, Tannerella forsythensis, Campylobacter rectus, Eikenella corrodens, Peptostreptococcus micros, Aggregatibacter actinomycetemcomitans* and tar-resistant *S. aureus* infections were present in most smokers[65-67]. These infectious agents can activate inflammatory molecules; which mainly involves an increase in the production of TNF α, IL-1β and IL-6[61]. Therefore, the increase in free radical exposure, reduced nutrient metabolism, tumour-promoting enzyme activation, blockage of detoxifying enzyme, change in the hormone status and carcinogenic components from alcohol or smoke may promote cancer through dysbiosis in the oral cavity.

Dietary changes may induce carcinogenesis through microbial activity in intestines. For example, protein-rich diet will increase protein fermentation and production of amino acid derivatives in the intestine. Some of the amino acid derivatives such as branched-chain fatty acids and phenylacetic acids are produced by bacteria belonging to the phylum Bacteriodetes and Firmicutes[68]. The overproduction of these amino acid derivatives leads to the nitrosation of these products leading to Liposuction of N-nitroso compounds. These compounds are potential carcinogens; and may damage DNA through alkylation and cause mutations[69]. Red meat consumption also modulates the microenvironment in the intestinal region. The tumour-associated microorganism *F. nucleatum* produces hydrogen sulphide in the presence of red meat which cause DNA damage in the colonic epithelium and the cells surrounding the tumour aid in the tumour formation and progression[70]. The polyamines are organic molecules derived from the arginine produced in the host tissues; these play vital role in maintaining the integrity of cell membrane and synthesis of nucleic acids[71]. Protein-rich diet will lead to the activation of protein fermenting microbiota and an increase in their number. This alteration causes overproduction of polyamines not only from the host tissues but also from gut microbial population[72] such as *B. fragilis, Salmonella enterica, H. pylori, Streptococcus pneumoniae, Shigella flexneri, etc*. The catabolism of polyamines produces oxidative stress and DNA damage in the intestinal mucosa[73].

Future research and therapeutic applications of the microbiome in cancer

Changes in microbial composition tend to disturb the cell microenvironment leading to an increase in proliferation of a particular species of microorganism. These alterations may cause DNA damage or tumour development, but in some exceptional cases, it might inhibit tumour as well[74] . The microbiome has the ability to enhance the efficacy of therapeutic treatments by modulation of host immune system. Therefore, microbiome does not always act to promote tumour formation but also play a vital and unique role to suppress tumour development with the aid of certain composition of microbes[75,76]. The tumour cells adapt certain immune checkpoint pathways to desensitize the host immune system against T cell and the antibody attack[77]. The immune checkpoint pathways are a self-defensive mechanism of the host to prevent autoimmune disorders. If these pathways are inhibited in tumour, the immune system can attack the tumour cells without any resistance[78]. The bacterial species of the commensal microbiome, *Bifidobacterium* strengthen the immunity against the tumour and intensify the activity of host dendritic cells[77]. It also suggests the manipulation of gut microbiome to enhance the efficiency of cancer immune therapy[77] (Table I).

Colorectal cancer shows high permeability towards inflammatory cytokine-producing cells. These tumour-infiltrating cells are able to activate intracellular pathways which help the colorectal tumour cells to grow[79]. The spent medium from the culture of tumour-infiltrating leukocytes has been shown to enhance the growth of colorectal carcinoma cell lines by activation of intracellular pathways and transcription factors such as STAT 3 and NF-kB[79]. T helper 17 cells from the tumour infiltrating leukocytes produce a large number of pro-inflammatory cytokines (IL-17A, IL-17F, IL-21 and IL-22), TNF-α and IL-6. These cytokines increase proliferation of colorectal

Table. Involvement of microbial dysbiosis in carcinogenesis

Tumour site	Microbial dysbiosis	Carcinogenic mechanism	References
Skin	An increase in *Staphylococcus aureus*, while a decrease in abundance of *S. epidermidis*	More likely to develop atopic dermatitis and non-melanoma skin cancer or basal cell carcinomas	15,16
	Population of flagellated bacteria like *Escherichia coli, Psuedomonas aeruginosa*, *Shigella* are increases	Flagellin-mediated TLR 5 activation and inflammatory responses	17,18,19
Oral cavity including lips, gingivae, teeth, hard palate, cheek mucosa, mobile tongue and floor of the mouth	Over proliferation of *Streptococcus anginosus*	DNA damage through overproduction of nitric oxide	26
	Increased population of *Porphyromonas gingivalis*, *Fusobacterium nucleatum*	Produces reactive oxygen species and nitrogen species which interact with bacterial or viral particles to form peroxynitrite leads to DNA damage	27
	Overproliferation of *S. anginosus*, *F. nucleatum*	Infection causes chronic periodontitis which leads to chronic inflammation, high risk to become pre-malignant lesions	27-30
	Neisseria, Streptococci and some other gram-negative microorganisms and yeast in presence of alcohol	Produces an extremely high amount of alcohol dehydrogenase enzyme and acetaldehyde which is, genotoxic and mutagenic	61-63
Gastrointestinal tract	Elevated level of *F. nucleatum*	FadA adhesion mechanism, also promotes cell proliferation with oncogene activation	34-36
	Abnormal proliferation of *Enterococcus faecalis*	Produces extracellular superoxide, which induces chromosomal instability, leads to colorectal cancer	40,41
Ovary, fallopian tube, uterus, cervix, vagina and vulva	Elevated level of *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum*	Through chronic inflammation and immunomodulation	45,48,49

TLR-5, toll like receptor 5

cancer cells[80]. The gut microbiome can shift through intake of an oral probiotic, which contains a unique probiotic mixture enriched with certain beneficial bacterial genera like, *Alistipes, Butyricimonas, Mucispirillum, Oscillibacter, Parabacteroides, Paraprevotella* and *Prevotella*[81]. Presence of these microorganisms downregulates the Th 17 cell infiltration and presence of inflammatory cytokines in the tumour. The metabolites released from the probiotic bacterial community inhibit the tumour-promoting activity of immune cells by activate the polarization of anti-inflammatory Type 1 regulatory T cells (Treg/Tr1) and promote their differentiation in the gut region[82]. Experiments conducted on hepatocellular carcinoma cell lines showed that these probiotic infused therapies might reduce tumour volume up to 40 per cent when compared to control[81].

As mentioned earlier the interaction between the tumour-associated immune cells and the microbiota induce inflammation, which contributes to the development of cancer. Therefore, the removal of the microorganism from the tumour surface may greatly reduce the production of a pro-inflammatory cytokine by the tumour-associated immune cells[16]. CpG-oligonucleotide immunotherapy and platinum chemotherapy are common treatment methodologies followed for gastrointestinal cancers[83].

Conclusion

The unique environment present in the human body promotes a specific composition of microbes to grow and contribute distinct functions. Through mutual interactions, each and every microorganism contributes toward the proper functioning of the human body. The diversity and relative abundance of the microbiome vary from site to site. Therefore, alterations in the microbiome can be uncovered only through comparative studies between normal and disease conditions. The microbiota may possibly give rise to cancer through the inflammation mediated mechanisms such as modulation of immune responses, or production of carcinogenic metabolites and genotoxins, or through activation of cell proliferative signalling pathway. The identification of these molecular mechanisms of carcinogenesis will aid to

improve treatment strategies. Along with new improved cancer therapies favourable microbiota inducing diet, anti-inflammatory drugs administration, prebiotic or probiotic treatment, transplantation of microbiome, administration of genotoxin neutralizing agents may aid in improving treatment outcomes.

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