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Bacterial biofilm in colorectal cancer: What is the real mechanism of action?

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ABSTRACT

Human colorectal cancer is the third most common cancer around the world. Colorectal cancer has various risk factors, but current works have bolded a significant activity for the microbiota of the human colon in the development of this disease. Bacterial biofilm has been mediated to non-malignant pathologies like inflammatory bowel disease but has not been fully documented in the setting of colorectal cancer. The investigation has currently found that bacterial biofilm is mediated to colon cancer in the human and linked to the location of human cancer, with almost all right-sided adenomas of colon cancers possessing bacterial biofilm, whilst left-sided cancer is rarely biofilm positive. The profound comprehension of the changes in colorectal cancer can provide interesting novel concepts for anticancer treatments. In this review, we will summarize and examine the new knowledge about the links between colorectal cancer and bacterial biofilm.

1. Introduction

Colorectal cancer is the third common cancer in males and the second common cancer in females, and it was 1.8 million new instances in the current year in the world [1–3]. It is the second leading cause of cancer deaths worldwide [4]. Approximately 55% of the colorectal cancer cases happen in developed countries [5]. In the industrialized countries, the lifetime risk of developing colorectal cancer is up to 5%, and the lifetime risk of developing an adenoma, a non-cancerous colon tumor that could make expanse into colorectal cancer, is 20% [5]. Despite tremendous development in the treatment of diseases, colorectal cancer is still a serious health problem in developed countries [6]. Various risk factors proposed for colorectal cancer, but currently have bolded a significant activity for the colon microbiota in the development of this disease [5,7–11]. Bacterial biofilm has been associated with non-malignant pathologies like Inflammatory Bowel Disease (IBD) but has not been fully demonstrated in the setting of colon cancer [12]. The investigation has found that bacterial biofilm is mediated to human colon cancer and linked to the location of cancer, with almost all right-sided adenomas of human colon and cancers possessing biofilm, whilst left-sided cancer is rarely biofilm positive [13]. Colorectal cancer is raising in humans younger than 50 years and is mediated by specific dietary agents and eating regimes that influence the human gut microbiota [14]. Hence, there is a chance for prevention, diagnostics as well as therapeutics based on the microbiota in colorectal cancer.

In normal situations, the human colon is enveloped by a mucosal barrier that separates the microbiome from direct contact with the colonic epithelium of the host [15]. The gap of this supportive mucus barrier with resulting raised contact between the colonic epithelial and microbiota has been suggested as a significant primary step in inciting modifications in the biology of cells and inflammation that cause IBD [16–18]. Bacterial community relationships with raised access to the underlying epithelium are predicted to modify and thereby change bacterial composition and activity and mostly triggering the formation

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of a biofilm [19]. A biofilm is described as associations of bacterial societies enclosed in an exo-polymeric matrix that binds to the biotic and abiotic surfaces [20,21]. The biofilm that invades the mucus layer of the human colon and goes to direct connections with epithelial mucosa shows a pathologic form [22]. Recently, the model that has been proposed for bacteria-stimulated carcinogenesis shows how the microbiome, bacterium to bacterium and bacterium to host interplays, involves in colorectal cancer [10,23–25]. However, the process by which the microbiota of human intestine interplay with themselves and the host to trigger and the progression of colorectal cancer remains mostly unknown. It is becoming evident that these colorectal cancer-eliciting interplays have a role in formation and spatial compositions of multi-bacterial societies in higher-order composition so-called biofilm [5,10,12,13]. The possible processes comprise damages of epithelial cells, chronic inflammation, and bacterial carcinogens, and so on [26,27]. In this review, we will summarize and discuss the relevance of bacterial biofilm with colorectal cancer in detail.

2. Microbiota of colon and bacterial biofilm

A microbiome is the collection of genomes from all the organisms found in a particular environment [28]. Humans, plants, and other animals all have microbiomes; these can be generalized to their entire organism or broken down into specific microbiomes for different locations on them [28]. Microbiota, on the other hand, usually refers to specific organisms that are found within a specific environment [28]. Microbiota can refer to all the organisms found in an environment, including bacteria, viruses, and fungi [28]. This means that there are localized differences in the microbiota of each person, depending on where in the body the microbiota is collected from.

The human colon comprises trillions of organisms that are separated from the human colonic epithelial cells by a dense layer of mucus [29]. This layer enhances tolerance to various foreign antigens by restricting bacterial–epithelial impact and, therefore, mucosal inflammatory reactions. In contrast, bacteria can breach into the human mucus layer of the colon with the formation of a biofilm to develop chronic mucosal inflammation [29]. The colon microbiota is the set of organisms include bacteria, viruses, protozoa, fungi as well as helminths, which reside in the host colon [30]. They are becoming a crucial property of health and disease. Now, achieving to the genomic findings of host cells and of microbiota is more affordable. The main challenge is how to integrate microbiota findings into precision medicine procedure for the prevention, diagnosis as well as treatment of disorders like cancer [30].

Traditionally, the attempts have focused on connecting particular bacterial factors and their respective toxins to colorectal cancer [31]. It has led to the characterization of putative bacterial oncogenic drivers of colorectal cancer, for instance, enterotoxigenic *Bacteroides fragilis* bacterium producing the *B. fragilis toxin* (BFT), *Escherichia coli* having the polyketide synthase (pks) encoding the genes need to form the genotoxin's colibactin, and *Fusobacterium nucleatum* bacterium containing the Fusobacterium-adhesin A (FadA) [31]. These bacteria have been connected via epidemiological evaluations with human colorectal cancer and have been found to trigger colon tumors in genetically susceptible *in vivo* models [31]. The identification that biofilm communities can harbor oncogenic risk factors offers a starting point for various novel lines of the survey that can yield further details in terms of the powerful tumorigenic involvement of the human microbiota to initiate and progress of colorectal cancer.

Biofilm is a bacterial community established in the self-produced extracellular substances that include up to 80% of bacterial associated infections [31]. During the formation of biofilm, the bacterial cells undergo the shifts of planktonic forms to the aggregated forms that are buried in an extracellular polymeric substance (EPS) [32]. During these shifts, single bacterial cells attached to a substratum. This attachment is reversible and, then, goes irreversible once the bacteria beginning to produce EPS including exopolysaccharide, extracellular DNA (eDNA) and the protein [33]. In contrast to intracellular DNA, which is the DNA located within cell membranes, eDNA represents the DNA located outside the cells [34].

Biofilm is densely packed bacterial aggregates, embedded in a self-formed extracellular matrix, that are more tolerant to immune clearance and antibacterial drugs [32,35]. The bacterial cells are thinned inside the biofilm and produce EPS, which accounts for up to 90% of their biomass [32]. The matrix of biofilm as a fixing framework is formed of EPS along with various carbohydrate-binding bacterial proteins and eDNA. Nutrients are hooked by the embedded bacterial cells in the biofilm matrix and, also, water is held [36]. The structure of EPS is changed in response to the variations in availability of various micro-nutrient by secretion of specific enzymes from bacterial cells, therefore tailoring the production of bacterial biofilm to the more certain environments [37]. The formation of biofilm is one of the strategies that organisms apply for persistence in humans and the progression of the disease [38].

Ecological limitations in the intestine favor the formation of biofilm [13]. Bacterial biofilm is involved in a chronic infection that is not simple to eliminate and appear to be a significant etiological agent in infectious disease, particularly cystic fibrosis, and endocarditis disease [39,40]. In terms of the later, the attachment of Streptococci to the extracellular matrix proteins (EMPs) of human endothelial cells and then the production of biofilm has been contributed to endocarditis [40]. Currently, biofilm has been connected to the initiation and development of colorectal cancer, usually in the right colon of humans (determined as proximal host colon to the hepatic flexure) [13]. Indeed, biofilm is much more common in tissue samples of colorectal individuals in comparison with normal controls [13].

Interplays between prokaryotes and eukaryotes are usual events. These interplays can influence the metabolism of the encountering members in various paths, resulting in neutral, beneficial, and/or harmful outputs for the members [41]. For example, long neutral or beneficial metabolic communications happen between mammalians and their gastrointestinal tract numerous microbiome [41]. Nevertheless, these beneficial communications are less permanent and can be disturbed by various agents including bacterial pathogens that form a biofilm. Metabolic communication happening in eukaryotic cells subsequent acute various biofilm-associated infections by pathogens are finally often conflicting. As the host cells effort to remove the biofilm while the pathogens attempt to benefit from host cell metabolites and micro-nutrients and simultaneously follow their biosynthetic and bioenergetic requirements so damaging the host cells [42,43]. Repeated metabolic shifts can, therefore, happen on the pathogens and on the host as well, during the biofilm-associated infections [10]. The explanations of host metabolic changes via the bacterial biofilms are of massive importance for the comprehension of biofilm pathogenesis.

An appearing probability is that the biofilm can include various bacterial species, instead of solely a single invading bacterium provide colonization with other pathogenic, and can cause raised inflammatory reactions and the formation of genotoxic compounds derived from the bacteria [44]. Accordingly, Fusobacterial spp. a dominant polybacterial biofilm was found to abundantly exists in tumor samples from individuals with colorectal cancer and adenoma but not in paired tumor-free samples (Fig. 1) [13]. Biofilm of driver bacteria can form new ecological microenvironments for passenger bacteria in the development of colorectal cancer, finally out-competing the driver bacteria [44]. Based on the carcinoma-adenoma sequence model suggested by Fearon et al. [45], bacterial biofilms can be regarded as the independent drivers at a primary phase of the carcinogenesis of colorectal cancer, before the malignant conversion from host adenoma to host carcinoma [44].

Numerous mechanisms mediating the role of bacterial biofilm in driving the colorectal cancer process have been suggested [10,13]. These mechanisms comprise the existence of diminished rates of epithelial cadherin (E-cadherin) of intestinal crypts, raised permeability of

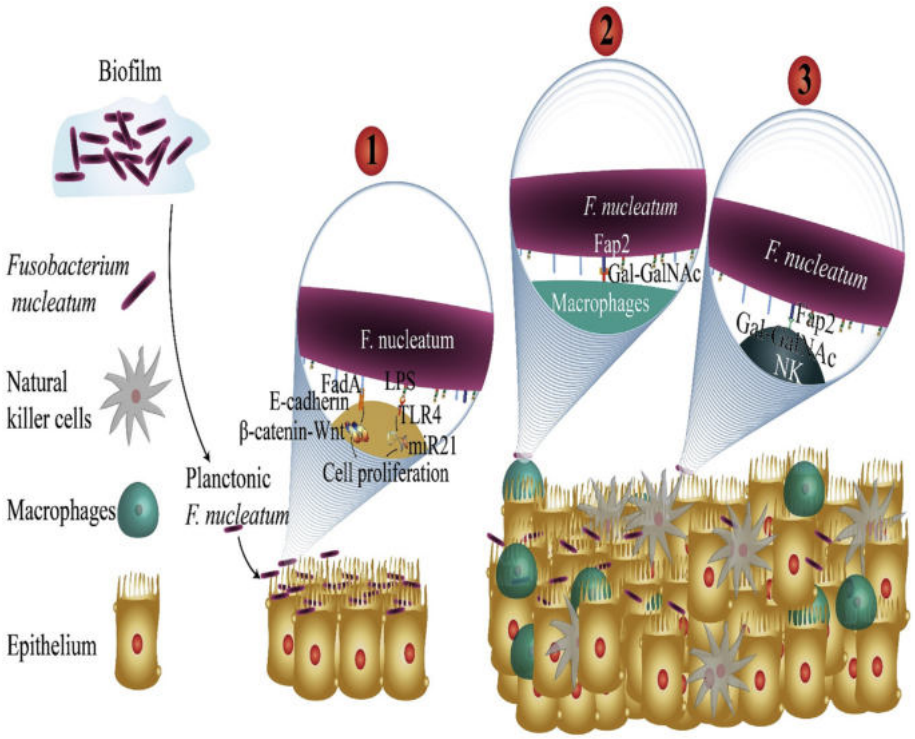

Fig. 1. The function of *Fusobacterium nucleatum* biofilm on colorectal cancer. After *F. nucleatum* cells detachment of biofilm, they are able to increase the cell proliferation in host cells *via* distinct mechanisms including1) the binding of FadA adhesion to E-cadherin which can stimulate the Wnt and β-catenin signaling pathways, and 2) stimulation of TLR4 and NF-κB which enable to cause the increased formation of the oncogenic miR-21. *F. nucleatum* subsequent the tumor microenvironment has developed could reach to the Gal-Gal NAC-forming tumor cells *via* Fap2. *F. nucleatum* cells functionally change the tumor microenvironment by the impact on the host myeloid cells and blocking anti-tumoral immune reactions of natural killers. A Normal host epithelium; B, Hyper-proliferation of host C; Tumor formation; LPS, lipopolysaccharide; NK, natural killer; TLR4, Toll-like receptor4; TIGIT, T Cell Immunoreceptor With Ig And ITIM Domains.

intestinal, the formation of polyamine metabolites and subsequently acetylation, and prompted stimulation of Interleukin-6/Signal transducer and activator of transcription 3 (IL-6/STAT3) signaling [10,13]. To trigger colorectal cancer carcinogenesis, bacterial pathogens may require the biofilm formation. Biofilm is not carcinogenic alone but just in terms of unique invasive pathogens, particularly Fusobacteria spp [13]. According to this, a biofilm has been found in the baboon, rat as well as the non-tumorous gut of human using electron microscopy investigation [46]. Also, one work found that a biofilm can be observed in the colon of normal murine, and a biofilm has been collected from healthy people by colonoscopy [47]. Additionally, colonoscopic biopsy specimen from healthy people has revealed that thin a biofilm in the mucosa consists of relatively harmless bacteria, especially Enterobacteriaceae, Bacteroidetes as well as Lachnospiraceae in the right host colon, and Lachnospiraceae as well as Bacteroidetes, but notably, no Fusobacterium, in the left host colon [13]. Most of these bacterial associations found here in normal mucosa are commensal bacterial spp. devoid of invasive potential [13]. Hence, the invasive capacity of biofilm-producing bacteria could be involved in colorectal cancer pathogenesis. To confirm this assumption, although Fusobacteria is a relatively prevalent and innocuous opportunistic biofilm-producing bacterial pathogen in the human oral cavity, in the intestine, biofilm could also stimulate severe inflammations [48,49].

Also, co-compression of *F. nucleatum* cells provides colonization with other pathogenic species in the bacterial biofilms, proposing that the production of a biofilm can indeed offer a new ecological microenvironment [50]. *F. nucleatum* cells can play as a powerful agent in the processing of bacterial biofilm production. For instance, the *in vitro* culture of a biofilm has found that this bacterium supplies a microenvironment for attachment and growth of *Tannerella forsythia* cells because *F. nucleatum* cells form a favorable niche for obligate anaerobe bacteria [51]. Therefore, *F. nucleatum* can recruit other species into biofilms. Campylobacter spp. like *Campylobacter curvus*, *Campylobacter concisus* as well as *Campylobacter rectus* are innocuous bacterial colonizers of the oral cavity of humans but are importantly mediated colonic and esophageal adenocarcinoma [52]. Many oral strains of *C. concisus* emerge to form zonula occludens toxin (ZOT) to stimulate cytoskeletal changes and to disconnect tight junctions (TJs) of human intestinal epithelial, enhancing bacterial translocation as well as inflammation [53]. Also, Campylobacter species are able to produce a biofilm, for instance, *Campylobacter showae* co-localize with species of Leptotrichia and Fusobacterium in colorectal tissues of human and can raise the risk of IBD [52,54,55]. Leptotrichia has been observed to be extremely plentiful in the human stomach with a high risk of gastric cancer rather than humans from low-risk populations, proposed that Leptotrichia species have the ability to stimulate intestinal cancer in human [56]. Therefore, anaerobic bacteria like Campylobacter, Fusobacterium as well as Leptotrichia emerges to be significantly mediated colorectal cancer [54]. Also, there is a link between *Streptococcus gallolyticus* and colorectal cancer. This bacterium has been found in 20%–50% of human colon tumor samples, while 5% of samples isolated from normal humans showed this organism [57]. Current works have found that Streptococci have attachment potential and the capability to produce biofilms [40,58]. Overall, these views indicated that the bacterium possessing co-aggregation and invasion traits can be needed for the production of tumor-enhancing biofilms.

3. Increased host–bacterium interplays in human colorectal cancer by biofilm

Biofilm could involve raising the permeability of intestine and enhance the barrier activity losses induced by the bacteria which, in turn, are of the most significant primary pathophysiologic changes in carcinogenesis of colorectal cancer in humans [59]. There are lines of the documents show the bacterial biofilm-enhanced barrier-loss notion. The former, pathogen invasions exist in full biofilm positive colorectal tumors in humans, including adenomas and colorectal cancer, but this invasiveness does not exist in biofilm negative colon tumors [13]. Latter, imaging by fluorescence in situ hybridization and scanning electron microscope has revealed that a dense poly-bacterial biofilm could be found in all right-sided tumors in the colon of humans, but few bacterial biofilms have been detected in left-sided human colorectal tumors [13]. Patients with right-sided colorectal cancer usually exist worse clinical effects compared to individuals with left-sided colorectal

cancer, which can be associated with this event [26].

Biofilm allows bacterial cells to live in next proximity to the host intestinal epithelial barriers, a significant situation for pathogen invasion and stimulation for the further inflammatory reactions [13,18,60]. Loss of intestinal barriers in humans also emerges to aggravate bacterial dysbiosis because this failure inhibited bacterial binding to human epithelial cells. The enhanced entry of bacterial components into host epithelial cells can subsequently be involved in the formation of a tumor-enhancing environment, like activating T helper 17 (Th17) immune reactions and favoring the initiation of colorectal cancer [13,60].

4. Biofilm and enhanced genotoxicity in human colorectal cancer

Of the probable process by which bacterial biofilm could enhance oncological disorders, genotoxic stress outcomes from bacterial toxins have shown the most document connections in comparison to transformation alone. For example, numerous bacteria generate different toxins, of which Cytolethal Distending Toxin (CDT) and BFT involve in genotoxicity and the initiation of colorectal cancer [61–65]. BFT molecules (formed by Enterotoxigenic *B. fragilis*) are the genotoxins that indirectly enhance the damage of DNA [63,66]. For instance, the BFTs in T84 and HT29/C1 colonic epithelial cells, *in vitro*, have been found to cause up-regulation of spermine oxidase molecules to trigger the formation of reactive oxygen species (ROS) [66]. It has been proposed that raised reactive oxygen species formation stimulates the Nucleotide oligomerization domain (NOD)-like receptor containing pyrin domain 3 (NLRP3) inflammasomes, a crucial activator of innate immune reactions that can more trigger the damage of DNA [67].

It has been suggested that BFT cleaves the E-cadherin (an intercellular adhesion molecule) of the colonic epithelial cell lines (for example, C1/HT29, Human colonic epithelial cells), so including intestinal barrier activity [68,69]. The intestinal barrier deterioration can cause raised leakage of bacterial components that can involve in colonic adenoma (pre-malignant lesions) [60]. Subsequent, as found in Mouse Model of Colonic Adenoma-Carcinoma Progression Based on Somatic Apc Inactivation (CPC-APC) murine *in vivo*, this can cause the Interleukin-23 (IL-23) and Interleukin-17 (IL-17)-contributed inflammatory reactions, resulting in damage of DNA in cells, and finally stimulate tumor production [60]. Especially, biofilms of *B. fragilis* are found to be the key characteristics of the IBD, of which the tumor-enhancing impacts of persistent intestinal inflammation of humans have now been detected and are best-defined [12]. Actually, over 60% of the biofilm mass collected from IBD patients has been ascribed to the Bacteroides spp [12]. Additionally, the production rate of biofilm associations, *in vitro*, found from IBD patients is significantly further than of samples taken from humans either with no inflammation or self-limiting colitis [12]. It is thus well-founded to suggest that a biofilm of *B. fragilis* is potent to enhance intestinal inflammation and colorectal cancer. It seems that multi-bacterial biofilms can theoretically change the intestinal tumor niches in many routs (like genotoxicity), and boost the expansion of human colon cancer.

5. Impacts of biofilm on metabolism in colorectal cancer

Metabolic interactions of bacteria and hosts can indirectly or directly provoke the progression of colorectal cancer stimulated by bacteria. Increasing documents have linked the microbiota of intestine to the control of many metabolic routs of exogenous and endogenous products, like biosynthesis of secondary bile acids, catabolism of polyamines. They also stimulated the carcinogens which in turn are mediated enhanced risk for numerous cancers in humans, for example, esophageal cancer, colorectal cancer as well as liver cancer [10,63,70–72]. As a good instance, deoxycholic acid emerges to be the most significant endogenous metabolite mediated colorectal cancer carcinogenesis in Apc Min/positive murine, *in vivo*, as well as in colon biopsies of human [71,73]. Deoxycholic acid molecules could act as the naturally-occurring carcinogens that trigger oncogenic changes in the epithelium of digestive tract in human because deoxycholic acid molecules have been found to stimulate DNA damages, oxidative stress as well as genomic instability in biopsy of esophageal host tissues and colonic cancer cells in epithelial human, and *in vivo* mouse models [71,74]. Additionally, deoxycholic acid molecules enhance the proliferation of tumor cells and suppress apoptosis by inducing Wnt signaling in the murine models [73]. Because biofilms can conceivably offer a very-efficient interface for dehydroxylation and deconjugation of bile acids, the cell of the epithelium covered by bacterial biofilm is exposed to very further rates of secondary bile acid molecules. In addition, such biofilms can also be a pool of nitrosamine and hydrogen sulfide (H2S) that have been demonstrated to be carcinogenic and genotoxic *in vivo* models, in colon cancer cells of human (HCT116, HT29, and SW480) as well, by stimulating damages of DNA and instability of genome [75,76]. Hence, it has proposed that bacterial metabolism in biofilm is involve in the carcinogenic capability of the underlying host epithelium.

By comparing biofilm and non-adherent bacteria, the higher rate of butyrate is formed by non-adherent bacterial aggregations show that preferable bacterial societies with the higher-order composition (biofilm) can show the altered formation of short-chain fatty acids like butyrate [77]. Fusobacterium-dominant biofilms can diminish the formation of short-chain fatty acids and might enhance the colorectal tumorigenesis. Nevertheless, short-chain fatty acids derived from bacteria like butyrate can also enhance the hyper-proliferation and sensitize epithelial cells of the colon to transformation (in mismatch repair-deficient Apc Min/positive mice model) as well [78]. Comprehension of the action of short-chain fatty acids in maintaining host intestinal epithelium or inducing colorectal cancer remains limited and modifications of these metabolites by bacterial biofilm certainly need more evaluation.

Documents show that a biofilm may involve in humans colorectal cancer via biosynthesis and acetylation of polyamine [10]. Multispecies biofilms produce spermidine/spermine N 1-acetyltransferase enzyme, which is required for acetylation of polyamine [10]. Therefore, the significance of this view bolded that demonstrating spermidine/spermine N 1-acetyltransferase in humans is not important for cancer development. Hence the expression of bacterial spermidine/spermine N 1-acetyltransferase may make a diversity in this regard [10]. Acetylated polyamine molecules are significantly increased in biofilm-covered host colon cancer. Moreover, paired healthy tissues compared to host colonic tissue devoid of bacterial biofilm, show that bacterial biofilm raises acetylation and catabolism of polyamine, then inducing undesirable proliferation of cells as well as the growth of cancer [63,79]. Additionally, enhanced rates of acetylated polyamine metabolite molecules, like N 1-acetylspermidine, N 1-acetylspermine, and particularly N1, N 12-diacetylspermine, are found in human colorectal cancer in comparison with matched non-tumor tissues from similar individuals [10]. In agreement, in human colonic cancer tissue samples, polyamine metabolite molecules at the mucosal margin, where the formation of bacterial biofilms starts, have been shown to form stronger mode to the center of the host cancer cell nest [10]. These data further showed that bacterial biofilms can be the most significant pool of acetylated polyamines [10]. Moreover, targeted metabolomic evaluations found that there are not any acetylated polyamine metabolites in bacterial biofilm-negative healthy colonic biopsies of the human. Hence, these findings more support the notion that the existence of polybacterial biofilm can be putatively associated with the growth of tumors in the human colon via a process involving raised rates of acetylated polyamine metabolites [10,10,44].

6. Role of Interleukin 17 (IL-17) in Colorectal cancer

The actions and locations of human immune reactions in the colorectal cancer microenvironment are very heterogeneous and complex

[80]. Reactions associated with Th1 toward established colorectal cancer are involved in better effects of patients, whereas responses associated with Th17 and formation of IL-17A resulted in worse effects of patients [80]. Cancers that grow in the mouse models of colorectal cancer are sometimes invasive and vary in some paths from human colorectal tumors. Nevertheless, these mouse models have been applied to survey the mechanisms by which IL-17A and Th17 boosting the trigger and growth of colorectal cancer, which shows to contribute to their direct impacts on colon epithelial. Particular parts of the colonic microbiota can enhance the formation of IL-17A and IL17A-forming cells, function in the colonic mucosa to boost carcinogenesis [80]. Raising the comprehension of the interplays between the mucosal human immune reaction and the colonic microbiota, the activities of IL-17 and Th17 in these interplays, and understanding how this mechanism is changed during the colon carcinogenesis, all can lead in developing new approaches for preventing and treating human colorectal cancer.

Colorectal cancer is a late-stage disorder, whereas many *in vivo* models that grow adenomas only representing primary disease. In patients, infiltrations of colorectal tumors by human immune cells are involved in the progression of tumor and clinical outcomes. Evaluation of the patient's effects is complex since they are influenced by disease phase, treatment, and genetic and environmental agents. A landmark investigation was done by Galon and colleagues indicated that the intra-tumor host adaptive immune reactions influence clinical outcomes, including decreased tumor recurrence [81]. These surveys have found that host adaptive immune reactions can enhance the regression of colorectal cancer [82]. Individuals with metastatic tumors with mismatch repair deficiency and further rates of cytotoxic T lymphocytes in the early tumor have a high level of reactions to checkpoint blockade-based immunotherapy [83,84]. Therefore, cytotoxic lymphocyte cells exist in the tumor that is suppressed by the various immunosuppressive ligands in the microenvironment may be stimulated by checkpoint blockade treatment to kill the host tumor cells [83,84]. Nevertheless, some individuals with metastatic colorectal cancer do not react to the immunotherapy, even though the early colon tumors are infiltrated by cytotoxic T lymphocytes. Other traits of the human immune infiltrate, containing the quality of the adaptive anti-tumor immune reactions can influence the progression of tumor and reaction to immunotherapy.

The adaptive human immune reactions to colorectal cancer have been well studied. The harmony between the patterns of gene expression of Th1 against Th17 cells inside tumors has been contributed to patient effects, but data have not sometimes been consistent [85–87]. Approximately two-thirds of early sporadic colorectal cancers were measured to have raised rate of IL-17A, whereas others have raised the rate of Interferon-gamma (IFN-γ) or a combination of IFN-γ and IL-17 formation [85,87,88]. Polymorphisms in human genes encoding IL-17E, IL-17A as well as IL-23 receptor, which are formed in the differentiation of Th17 cells, have been involved in the elevated risk of colorectal cancer and little outcomes [88,89]. These data offer documents that show Th17 cells and IL-17A involved in the progression and development of colorectal cancer [90]. Nevertheless, more researches are needed to well-defined the activities of IL-17A and other cytokines that can involve in the progression of colorectal tumors.

An *in vivo* model of colorectal cancer provides the document for the communication among adaptive and innate immune reactions and the microbiota of colon in tumorigenesis of colorectal disease [5]. It is not obvious which parts of the human microbiota and which mucosal host immune reactions can trigger sporadic and hereditary colorectal cancers. It is often mentioned that some bacteria *like F. nucleatum, Streptococcus gallolyticus*, pks-positive *E. coli*, ETBF as well as *Enterococcus faecalis* are involved in colorectal cancer [80]. Innate and adaptive human immune reactions have involved in colon carcinogenesis in murine colonized with ETBF cells, whereas myeloid populations involved in colorectal tumor production in mice colonized with *E. faecalis and F. nucleatum* [91,92]. Drewes and colleagues surveyed available sequences of 16S ribosomal RNA (16SrRNA) gene via a single computational pipeline and five bacteria involved in sporadic colorectal: *F. nucleatum, B. fragilis*, *Gemella morbilliform, Parvimonas micra and Peptostreptococcus stomatis* [93]. *F. nucleatum*, *G. morbilliform*, *P. stomatis* as well as *P. micra* are usually detected in the oral microbiota. Parts of the human oral microbiota have increasingly been mediated to colorectal cancer. Nevertheless, the impacts of these bacteria on tumorigenesis and mucosal immune reactions have not been evaluated in the mouse. Dejea and colleagues associated mucus-invasive biofilms to the sporadic colorectal cancer and tumors in individuals with familial adenomatous polyposis [13,94]. A polybacterial biofilm was found on sporadic host colorectal cancer and non-tumor host colon tissues from the same individuals and had a role in carcinogenic shifts in colon epithelial cells [13]. In individuals with familial adenomatous polyposis, a biofilm was detected on normal mucosa and nonmalignant colon polyps; over 50% of the bacterial population included pks positive *E. coli* and ETBF. Administration of azoxymethane in a C57Bl/6 mouse and co-colonized with pks positive *E. coli* and ETBF raised formation of IL-17A and increased the adherence of pks positive *E. coli* to the colonic mucosa mediated by ETBF was required for the development of colon tumor [94]. The notion that bacteria-stimulated IL-17A mucosal host adaptive immune reactions may be carcinogenic is protected by views that show gastritis mediated by *Helicobacter pylori* requires IL-17A [95]. It has been shown that segmented filamentous organism, a mouse bacterium, stimulates the formation of IL-17 in the small intestine of a mouse. The data led to the notion that particular bacteria are needed for stimulation of intestinal immune reactions mediated by IL-17 [96].

Nevertheless, a survey is doing to detect bacteria that stimulate IL-17 formation in the colon of humans beyond the primary association of IL-17A formation with the colonization of ETBF. In addition, Atarshi and colleagues found that reactions of Th17 in the host intestine with bacteria require physical contact between the intestinal epithelial and bacterial cells [97]. These data showed that responses mediated by the mucosal Th17 cell require molecular patterns mediated to bacterial cells and the physical vicinity of the bacterium to the host epithelial cells.

7. Role of the iron released from the biofilm

Bacterial pathogens have earned many mechanisms to escape from commensal-stimulated resistance and human immunity, mechanisms which in turn provide the bacteria with impressive virulence factors [98]. Formation and utilization of local luminal metabolites show key regulators of pathogen-commensal pathobiont interplays and are required for the selection of niche and as well as controlling disease and infection [99–101]. As microbiota dysbiosis is involved in shifts in the rates of host and bacterial metabolites, these, in turn, provide promise in our quest to find new therapeutic and biomarkers targets for disorders induced by dysbiosis of the microbiota.

Some members of Proteobacteria require to properly produce or incorporate metabolites, including those mediated by the iron-associated pyrimidine and purine metabolism, from their environment to efficiently persist and colonize in the intestine and/or to grow in the bloodstream [102,103]. Iron is an element that has a significant action in virulence and niche selection [98]. Current data show that bacterial genes encoding for the acquisition of iron (Yersinia bactin, chu operon) and utilization of propanediol (pdu-operon) are overproduced in Adhering Invasive *E. coli (*AIEC*)* [104]. Moreover, the formation of cellulose by AIEC involve in a promotion dependent on the iron of bacterial aggregation. It proposes that iron has indirect and direct impacts on the formation of biofilms for some bacterial species. Proteobacteria could thrive at the expense of other gut bacteria in an iron-rich environment. So, iron-acquisition offers a significant factor in bacterial virulence. Further, pathogens (like those from the Enterobacteriaceae) are known to show the increased capacity of iron uptake, but

information on human mucosal biofilm was lacking. Current data have now indicated that iron uptake is a critical process in conferring virulence to pathobionts dispersed by biofilms of microbiota in individuals with IBD.

8. Polybacterial biofilms as a stimulator of host pro-carcinogenic inflammatory reactions

The involvement of inflammation induced by bacterial cells in tumor growth is demonstrated in many documents [66,105,106]. Particularly, the IL-17/IL-23 signaling axis shows to be necessary for inflammation stimulated by the bacteria [60,107]. This route has been found to enhance the accumulation of granulocyte with antibacterial activity, but it could also cause DNA damages [25,60,106]. In addition, granulocyte is particularly prone to form pro-inflammatory cytokines like Interleukin-1 (IL-1), IL-6 as well as Interleukin-21 (IL-21) [25,60]. Also, there are studies that support activity for IL-17 in the expansion and progression of human colorectal cancer [60,107,108]. Accordingly, bacterial toxins like BFT, selectively enhance proinflammatory reactions dependent on IL-17/IL-23-, inducing colitis disorder in the mouse [23,24]. For instance, blockade of IL-23 and IL-17 receptors by blocking antibodies, suppress the colitis induced by ETBF in the Apc Min/positive murine, and only STAT3 molecules are stimulated subsequent colonization of ETBF [24]. In another case, depleting the intestinal bacterial flora in CPC-APC murine by three weeks of cocktail therapy with broad-spectrum drugs diminished the expression of IL-17A mRNA and reduced the activation of STAT3 in colonic tumor cells [60]. In addition, three months of drug therapy markedly decreased the size of colon tumors in CPC-APC mice in comparison with IL-23R-deficient CPC-APC murine, further showed that tumor-eliciting gut bacteria can enhance the pro-oncogenic signaling dependent to IL-23 [60]. Current works have found a significant positive connection between the increase of intra-tumor Fusobacterium cells and the rates of various inflammatory cytokines like IL-6 and TNF in human colorectal. Although no such a correlation was observed in the colonic mucosa of controls, it suggested that carcinogenesis induced by Fusobacteria can be involved in the capability of bacterial biofilm to trigger mucosal inflammation [109,110]. As a result, the stimulation of the immune reactions mediated by IL-17/IL-23 in the intestinal epithelium can be associated with the putative pro-oncogenic capability of bacterial biofilm. Increasing documents also proposed the notion that mucosal biofilm prompts the pro-oncogenic capability of bacterial dysbiosis by eliciting inflammatory reactions [12,13,111]. For example, the rates of mucosa-adherent pathogens, i.e., biofilm production, were shown to be significantly superior in biopsies from individuals with Crohn's disorder in comparison with non-inflammatory controls, like individuals with IBD and healthy individuals. Usually, all of the microbiota found in the host intestinal tract of IBD patients were bonded to the epithelium [12]. Microbiota of the human intestine is controlled by interplays between host immunity and bacterial activities, and they contributed to highly structured spatial compositions of bacteria linked to the specific immune reactions [12,13,111]. For example, highly-virulent Enterococcus (HVE) that colonize the human rectum and colon, produce a bacterial biofilm that supports organisms from reactive oxygen and enhances detoxification of hydrogen peroxide activity in IBD individuals [112]. Contrariwise, biofilms also increase the specific immune reactions that in turn can induce the development of cancer. As previously noted, biofilms can directly cause the stimulation of IL-6/STAT3 proinflammatory signaling in epithelial cells of the human intestine (a well-defined tumor-enhancing route). According to this, a current work found that phosphorylation rates of STAT3 and IL-6 were markedly increased in a non-tumor biopsy specimen from individuals with bacterial biofilm-positive colorectal cancer cells in comparison with normal mucosal cells from bacterial biofilm-negative colorectal cancer patients (Fig. 2) [13]. Additionally, IL-6 molecules were shown to be further formed in biofilm-covered human intestinal mucosa with STAT3 stimulation, even in healthy controls, showed that bacterial biofilm alone can enhance IL-6/STAT3 inflammatory signaling [13].

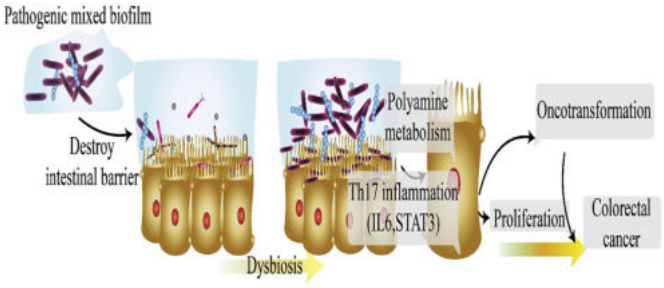

Fig. 2. The mixed biofilm in the inner colonic mucus of the host. Biofilm leads to raised gut permeability, redistribution of E-cadherin in host colonic cells as well as the loss of activity of intestinal barrier, subsequent, increasing intestinal dysbiosis. Host dysbiosis can favor raised growth of other opportunistic organisms. The pro-oncogenic activity of the bacterial biofilm, along with changes of polyamine metabolic and inflammation-mediated with Th17 causes the proliferation of host cells and aberrant tumor growth.

Other cases involved in the action of inflammation are amyloid fibril molecules. The concept that inflammation is contributed to amyloid pathogenesis was first noted in research on serum amyloid A [113,114]. Today, it is defined that this particular amyloidogenic expression is modulated by the inflammatory cytokine, IL-6, which results in enhanced rates of serum amyloid A [115]. These amyloid compositions constitute an important and common ingredient of mucosal bacterial biofilm and are formed by dominant intestinal bacteria belonging to the Bacteroidetes, Firmicutes as well as Proteobacteria [116–118]. Amyloid fibril molecules like curli could stimulate the signaling of the Toll-like receptor (TLR) in host immune cells [117,118]. Curli molecules, the powerful Toll-like receptor 1(TLR1)/Toll-like receptor 2(TLR2) ligand, have been found to stimulate a host response mediated to TLR2 in murine colitis, involving in inflammatory reactions contributed to IL-17A/Interleukin-22 (IL-22) [117–119]. In another study, by incubating naïve CD4 + T cells of the mouse with supernatant of dendritic cells derived from bone marrow pretreated with curli molecules *in vitro*, the formation of IL-17A and IL-22 could stimulate the differentiation of naïve CD4 + T lymphocytes into Th17 lymphocytes [118]. Overall, it is thus reasonable to propose that signaling dependent on pro-oncogenic Th17 can be enhanced by biofilm, cause the intestinal inflammation that, when is intense, can constitute a route by which a could be biofilm involve in colonic neoplasia in human.

9. Conclusion

Human intestinal bacterial biofilms and their compositions emerge to play a crucial activity in sustaining and triggering colorectal progression. The molecular process in the interplay between carcinogenic factors formed by bacteria and biofilm and host reactions in the initiation and progression of colorectal cancer is now emerging. However, it is evident that more studies will need to offer mechanistic details into their precise contribution to the process of human cancer. The relative credit of this phenomenon remains unknown, but with the appearance of screening programs for colorectal cancer, and the mediated possibilities for prospective works, fast progress in this regard seems likely. When considering the distinct properties of the spatial composition of microbiota in the distal and proximal colon tissues in humans (comprising normal and cancer tissues), significant questions must be increased on whether the invasive formation of bacterial biofilm preferentially exists in right colon cancer of human. In line with the unfavorable prognosis of human right-sided colorectal cancer, it has postulated that bacterial biofilm-positive colorectal tumors can cause worse clinical results associated with the bacterial biofilm-negative tumors given that bacterial biofilm aggregations can cause further

serious injuries and intestinal inflammation of epithelial tissue. Additionally, current emerging documents support this notion; acetylated spermine could be significantly decreased in resected tumor tissues from colorectal patients administering antibacterial drugs before their surgery in comparison with untreated colonic bacterial biofilm-covered cancer tissue specimens. It is, therefore, assumed that the initiation and progression of colorectal cancer arise as a result of the pro-oncogenic properties of bacterial biofilm of invasive bacterial pathogens. Preventive approaches aimed at the primary detection and suppression of such bacterial biofilm deposition can prove to be profitable for individuals with the risk of colorectal cancer. Moreover, specific drugs targeting biofilms can be used among numerous potential therapeutic protocols. Nevertheless, such approaches must be evaluated cautiously when attempting to target cancer tissues because they change the intestinal milieu of humans and its microbiota can cause deleterious consequences and metabolic imbalances for the host. Hence, there is a crucial need for models that more closely reflect the *in vivo* infection conditions. Indeed, the profound comprehension of the metabolic host cell reactions in colorectal cancer can provide novel interesting concepts for anti-cancer treatments.

In summary, these data suggest bacterial biofilm is an important factor contributing to colorectal cancer development. We anticipate that evaluating bacterial biofilms as well as the type of inflammatory reactions will provide a clearer picture of how bacteria contribute to the initiation and progression of colorectal cancer. Identifying candidate pro-carcinogenic bacterial species from human colon mucosal biofilms can enable earlier screening to predict patients at risk of developing colorectal cancer and allow the application of interventions to disrupt the progression of colon carcinogenesis.

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Declaration of competing interest

Nothing declared.

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