

Periodontitis, *Fusobacterium nucleatum*, and Colorectal Carcinoma. A Review

Michal STRAKA¹, Petra BORECOVÁ¹, Matej STRAKA¹

¹ Department of Dentistry, Faculty of Medicine, Slovak Medical University, Bratislava, Slovakia

Correspondence to: MUDr. Michal Straka, Ph.D.
Department of Dentistry, Faculty of Medicine, Slovak Medical University,
Bratislava, Slovakia, Limbová 14, 831 01 Bratislava, Slovakia
E-MAIL: mudrstraka@r3.roburnet.sk

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Abstract

Our review study addresses chronic periodontitis and its potential complications in the distal segments of the intestine and rectum. Subgingival colonization by gram-negative anaerobic bacteria such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Eikenella corrodens*, and *Fusobacterium nucleatum* may, through haematogenous dissemination into non-oral tissues and organs, cause severe systemic diseases. In connection with colorectal carcinoma, the third most frequently diagnosed malignant tumor, special attention has been focused on the anaerobic rod *Fusobacterium nucleatum*, one of the key periodontal pathogens involved in periodontal pocket infections. A growing amount of direct and indirect evidence supports its role in the development, progression, and persistence of colorectal carcinoma in the distal colon and rectum.

F. nucleatum possesses numerous virulence factors that underlie its remarkable infectious potential, not only within the oral cavity but also in the colonic environment, where they facilitate its integration into the dysbiotic microbiome and directly contribute to carcinogenesis in this region. Disruption of the physiological microbiota and colonization by *F. nucleatum* are now considered major drivers of malignant tumorigenesis in the distal colon. Several studies confirm the oral origin of *F. nucleatum* and its potential haematogenous spread into the intestinal microenvironment. Eradication of *F. nucleatum* from the colon is regarded as a crucial factor in achieving successful treatment outcomes for colorectal cancer (CRC). However, systemic administration of broad spectrum antibiotics adversely affects the composition of the normal gut microbiome, leading to microbial imbalance. For this reason, the elimination of *F. nucleatum* in the colon and rectum relies on a whole range of antibacterial agents that minimally disrupt the gut microbiota.

Our eradication strategy for *F. nucleatum* emphasizes close cooperation between dentists or periodontologists and gastroenterologists or oncologists, targeting high-risk populations: patients with IBD, colorectal adenomas ≥ 1 cm, multiple polyps, or first-degree relatives with CRC diagnosed before age 60. These at risk patients undergo dental evaluation for periodontitis and gingivitis by collaborating

dentists. Identified cases are treated using localized, comprehensive, and early eradication strategies targeting *F. nucleatum* and other periodontal pathogens within the periodontal pocket microenvironment. The primary objective of early interdisciplinary cooperation is to detect early stages of periodontitis with periodontal pocket depths of up to 4 mm. In such early forms of periodontitis, elimination of infection can be achieved through local approaches including scaling, deep scaling, and curettage, combined with the application of antibacterial solutions, varnishes, antimicrobial impregnated fibers, and, where appropriate, the use of periodontal lasers.

INTRODUCTION

Colorectal carcinoma (CRC) is the third most frequently diagnosed malignant tumor and the second most common cause of death in the current human population. In 2020, its worldwide prevalence was 1.9 million patients, and in the same year 0.9 million patients died as a consequence of the disease. Several pieces of information and evidence indicate that periodontal pathogenic microorganisms involved in the origin and maintenance of periodontitis may, to a considerable extent, participate in the etiopathogenesis of colorectal carcinogenesis (Idrissi Janati *et al.* 2022; Sung *et al.* 2021).

For the purpose of clarifying the association between chronic periodontal inflammations and colorectal carcinoma, several hundred studies of different validity and various focus have been carried out, from which the most important ones will be presented in this review study. The majority of studies on this issue present the participation of periodontal pathogens in the origin of colorectal carcinomas as a serious etiopathogenetic cofactor. However, to clarify some associations regarding the microbial burden of CRC lesions, it is necessary to investigate several groups of issues in order to at least partially shed light on this matter.

RESULTS OF EPIDEMIOLOGICAL STUDIES

To clarify the statistical associations between periodontitis and colorectal carcinoma (CRC), several studies have been carried out and presented. In an observational case-control study, 348 patients with a recent diagnosis of colorectal carcinoma were monitored and compared with a population of 310 control individuals of the same age and sex. After elimination of several etiological cofactors such as age, sex, BMI, diabetes mellitus, social status, genetic family burden of CRC, consumption of red meat, and sports activities, it was statistically found that patients with periodontal disease and diagnosis of CRC had the following score: RR -- 1.45; 95% CI 1.04–2.01; $p = 0.026$. These results can be interpreted as stating that in patients with periodontitis the incidence of a new CRC disease is 1.45 times higher

than in patients in the control group. These results support the hypothesis of a mutual association between periodontitis and CRC (Idrissi Janati *et al.* 2022).

In another comparative cross-sectional study, it was examined whether periodontitis may be a risk factor for the development of colorectal carcinoma. In the first group of 216 subjects with periodontitis, these patients had a clinically defined pocket ≥ 4 mm on one or more teeth. The control group consisted of 2288 patients without periodontitis. In the group of patients with periodontitis, the prevalence of CRC was significantly higher, by as much as 25%, while in the control group the prevalence of CRC was approximately half, 12.3%. Other risk factors in the multivariate analysis were smoking, age, waist circumference, and triglycerides, and the factors for advanced CRC were periodontitis, age, and family history of colorectal carcinoma. Periodontitis was associated with an increased risk of proximal CRC, with the following values obtained [OR 1.525; 95% confidence intervals (95% CI) 1.071–2.172], and of proximal advanced CRC (OR 2.671; 95% CI 1.088–6.560). These values can also be interpreted as stating that patients with periodontitis have a 1.525 times higher risk of developing CRC, while in advanced forms of CRC the risk in patients with periodontitis increases up to 2.671 (Kim *et al.* 2019).

On the above mentioned epidemiological studies, we illustrated the methodology and management of studies dealing with the associations between periodontitis and the occurrence of colorectal carcinomas. In a comprehensive evaluative multivariate analytical study by Chinese authors, research works from the years 1966 to 2020 were used, which were included in the databases Web of Science, PubMed, EMBASE, and the Cochrane Library. A total of 7 studies were included in the meta-analysis, and 9 studies were included in the narrative synthesis. The final statistical-epidemiological results of this extensive study confirmed the fact that periodontitis significantly increases the risk of colorectal carcinoma by 44% (RR 1.44; 95% CI 1.18–1.76; I^2 55.2%). However, for the assessment of causality or causal linkage between both diseases, further studies are still necessary (Li *et al.* 2021).

CAUSAL CONNECTIONS BETWEEN PERIODONTITIS AND CRC

Destructive forms of periodontitis represent a serious source of gram-negative bacterial pathogens organized in the subgingival biofilm. The dental microbial biofilm is an organized community of microorganisms that are attached and embedded in an extracellular polymeric matrix, adhering to various oral surfaces (Hobley *et al.* 2015). A particularly pathogenic structure is the subgingival microbial plaque, which is not easily accessible by means of common oral hygiene. In this complex ecosystem, which protects the bacteria contained within it, the most frequently detected periodontal pathogens

possess numerous serious virulence factors that enable their hematogenous spread and propagation into distant tissue and organ structures. Among the serious periodontal pathogens, it is necessary to mention especially *Porphyromonas gingivalis*, which is present in all clinical forms of periodontitis. Other periodontopathic bacteria include *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Eikenella corrodens*, and *Treponema denticola* (Colombo et al. 2015; Straka, 2016).

Fusobacterium nucleatum is associated with several non-oral diseases that are linked to destructive periodontitis, and its primary occurrence in these lesions is connected with systemic hematogenous propagation into other tissues and organs. In monitoring possible associations with other systemic diseases, it was most often detected in atherosclerotic/atheromatous vascular lesions, in 21 ruptured samples out of a total of 36 ruptured samples (56%) taken from intracranial aneurysms, together with other periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Treponema denticola* (Pyysalo et al. 2013). In atheromatous plaques it was detected in 50% of samples, while the most virulent periodontal pathogen *Porphyromonas gingivalis* was identified in 78.57% of samples (Figuro et al. 2011). Several studies report positive correlations between premature births of low-birth-weight infants and their findings in chorioamnionitis (Straka, 2011; Bohrer et al. 2012; Gauthier et al. 2011). It should be added, however, that providing references for all non-oral localizations and their possible pathogenetic effects exceeds the scope of this review study.

In monitoring possible oral microbial pathogens in neoplastic lesions of colorectal localization, the common opportunistic bacterium, but also a well-known periodontal pathogen, *Fusobacterium nucleatum*, was identified by several studies and research centers. *Fusobacterium nucleatum* is a Gram-negative, non-motile anaerobe with a conical shape and spindle-like tapered ends, regularly isolated from samples of subgingival plaque originating from various forms of gingivitis and periodontitis, mainly chronic forms. It constitutes up to one tenth of the total amount of isolated bacteria. Its occurrence has been detected in many types of clinical lesions, with its findings being associated with the depth of the periodontal pocket and its progression. Some proteins of its outer membrane are capable of inducing the cell death of human lymphocytes (Kaplan et al. 2010; Liu et al. 2014). From other oral diseases, it has been detected in endodontic periapical lesions (Siqueira et al. 2009; Rocas et al. 2011), in halitosis, oral cavity cancer, and in pulp inflammations (Chen et al. 2022). In the analysis of subgingival biofilm in 25 patients with chronic periodontitis and 29 patients with a healthy periodontium, the PCR method showed that *F. nucleatum* was present in all patients, and in healthy patients it was identified in as many as 86.21%

of samples, which means that *F. nucleatum* is an important opportunistic infection that, with the emergence of a pathological microenvironment in the form of a periodontal pocket, can transform into one of the main periodontal pathogens. Evidence of its high and increasing prevalence rises with the depth of the periodontal pocket, the severity of inflammatory changes, and the degree of progression of periodontitis (Guo et al. 2017; Chen et al. 2022).

F. nucleatum is an important component of oral biofilms, and its cumulative pathogenicity lies in the interaction between gram-positive and gram-negative bacteria during the formation of dental plaque (Chen et al. 2022). Its surface virulence factors in the form of adhesins FomA, Aid1, and RadD participate in the collective adhesive and colonization capacity and the mutual interconnectedness of bacteria in the biofilm (Chen et al. 2022; Guo et al. 2017), while the adhesin RadD of *F. nucleatum ssp. polymorphum* specifically binds with the surface SpaP of *Streptococcus mutans*, the main gram-positive bacterium of oral biofilms (Guo et al. 2017). A very significant virulence factor in the process of forming a pathological subgingival biofilm is its ability to aggregate with the most important periodontal pathogen *Porphyromonas gingivalis* through its virulence factors Fap2 and FomA. The capacity for co-aggregation with several periodontal pathogens in the terminal stages of subgingival biofilm formation significantly increases its pathogenic potential (Chen et al. 2022; Copenhagen-Glazer et al. 2015). From the mentioned virulence factors acting in the oral cavity, it is clear that *F. nucleatum* is a significant etiopathogenetic factor in the development of pathological dental plaque and periodontitis (Chen et al. 2022; Guo et al. 2017; Copenhagen-Glazer et al. 2015). *F. nucleatum* is capable of binding to many types of immunocompetent cells and substances and destroying them (Han, 2015). At this point, it is necessary to raise the question: Which virulence factors of *F. nucleatum* are responsible for its extraoral pathogenicity concentrated in the gastrointestinal tract and other extraoral tissues or organs? To answer this question, we must first provide some basic information about the bacterium itself and the possibilities of its transmission into the digestive system.

The inflammophilic pathobiont of *F. nucleatum* is associated in the human organism with various dysbiotic diseases in many localizations. The current taxonomy of the species *Fusobacterium nucleatum* recognizes four subspecies: *F. n. nucleatum*, *F. n. polymorphum*, *F. n. animalis*, and *F. n. vincentii*, with the following distribution in the oral cavity. The most common subspecies of *F. nucleatum* in dental plaque is *F. n. polymorphum*, which changes significantly in odontogenic abscesses where *F. n. animalis* dominates. The most studied subspecies, *F. n. nucleatum*, represents a numerically minor proportion in the oral cavity compared to the other subspecies. In sites with active

inflammation, *F. n. animalis* occurs most frequently (Han, 2015).

There is a considerable amount of valid evidence regarding the ability of periodontal pathogens to penetrate into circulation through transient bacteremias, whereby bacteria escaping into the bloodstream may become a source of complications in various systems, including the gastrointestinal tract and even neoplastic diseases (Kitamoto *et al.* 2020; Dewhirst, 2016; DiRienzo, 2014). Several bacterial species of both non-oral and oral origin have been associated with neoplastic diseases in the colon and colorectal carcinomas, and the bacterial theory of colorectal carcinogenesis has been the subject of many differently conceived and conducted studies. Specific bacteria were present as bacteremias in the early stages of neoplastic disease. A relatively strong body of evidence emerged from the detection and analysis of 13,000 diagnosed bacteremias in Hong Kong, where after one year an increased frequency of colorectal carcinoma was observed. Among several investigated non-oral bacteria, *Gemella morbillorum*, *Clostridium perfringens*, *Clostridium septicum*, and *S. gallolyticus* were identified, with statistically significant associations between prior bacteremia and CRC (compared with the control group) established for *C. septicum* and *C. perfringens*. Among oral bacteria, *Fusobacterium nucleatum* was identified within the bacteremias (Kwong *et al.* 2018). In the study of possible etiopathogenetic associations, it was found that *Streptococcus gallolyticus* and the oral periodontal pathogen *Fusobacterium nucleatum* are preferentially localized in neoplastic lesions, with *S. gallolyticus* strongly stimulating the hyperproduction of proinflammatory cytokines IL-1, IL-6, IL-8, and TNF-family cytokines, and inducing proliferation of colon cells in specific cell types (Kwong *et al.* 2018; Knippel & Sears, 2021; Boleij & Tjalsma, 2013).

The important periodontal pathogen *F. nucleatum* has been repeatedly identified in biopsies and samples of various forms and stages of Crohn's disease and ulcerative colitis, currently grouped under the disease known as IBD (Inflammatory Bowel Disease), which is now considered a significant risk factor for the development of colorectal carcinomas (Strauss *et al.* 2011; Tahara *et al.* 2014). *F. nucleatum* and other species of the genus *Fusobacterium* have been identified in tumor lesions together with several Gram-negative anaerobes that were found as components of the oral microbiome, including anaerobes from the genus *Campylobacter* (Strauss *et al.* 2011), which also occur in periodontal lesions as the species *Campylobacter rectus*. The presented and identified polymicrobial set from 130 colorectal carcinoma lesions induced a host inflammatory response in many pro-inflammatory markers and substances, with extremely high expression of the gene for the chemokine IL-8, which attracts a large number of pro-inflammatory cells into the lesions (Warren *et al.* 2013). It has been found that the

invasiveness of *F. nucleatum* varies markedly between different bacterial strains, and the course of the disease itself also significantly depends on its activity (Strauss *et al.* 2011). *F. nucleatum* has been and continues to be frequently identified in tumorous diseases of the colon and rectum. Some studies have raised a serious question formulated as follows: Is *Fusobacterium nucleatum* a random member of the pathogenic bacterial group, or is it a driver of tumorigenesis in the colon and rectum? (Han, 2015; Tjalsma *et al.* 2012).

Gut dysbiosis and *F. nucleatum* colonization

To answer the above-mentioned question, it is necessary to present several essential findings derived from variously designed studies. Although several groups of risk factors contribute to the onset and development of colorectal carcinomas, such as genetic predisposition, which accounts for 12 to 35% of risk factors (Dekker *et al.* 2019), one of the main risk factors is also the gut microbiome, or more precisely, gut dysbiosis. Different subspecies of *F. nucleatum* occur directly within tumors, most frequently in combinations of one or two subspecies, and the subspecies *F. n. animalis* has been most commonly identified intratumorally (Dekker *et al.* 2019; Ou *et al.* 2022). Increased amounts have also been detected in stool samples (Ou *et al.* 2022; Amitay *et al.* 2017). However, the total or elevated levels of *F. n.* in stool do not proportionally correspond to the increased amounts in the intestinal epithelial lining of the same individual (Flanagan *et al.* 2014). The gut microbiota, from the perspective of its local distribution within the intestine, can be divided into a portion associated or communicating with the mucosa and a portion located in the intestinal lumen, also referred to as luminal-fecal, while the mucosa-associated part has a closer and tighter relationship to colorectal carcinoma (Ou *et al.* 2022). The support of the onset and progression of the tumorous process in the colorectal region, mediated and maintained by *Fusobacterium nucleatum*, is reinforced primarily by the following facts, findings, and study results, which can be divided into several groups or categories.

Increased Amounts of *F. nucleatum* in Tumors

At the site of the tumor itself, the quantity and concentration of *F. n.* is higher than in other segments of healthy intestine, while the amount of *F. n.* decreases with increasing distance from the tumor (Flanagan *et al.* 2014; Yamaoka *et al.* 2018). In comparison, the levels of *F. n.* in tumor-altered tissue are relatively higher than in samples of mucosa from healthy individuals (Ou *et al.* 2022).

F. n. concentrations increase with the severity of CRC

Patients with advanced tumorous lesions exhibited a higher prevalence of *F. n.*, which was also confirmed in cases where CRC was classified into stages I–III (Ou *et al.* 2022; Amitay *et al.* 2017).

F. n. Colonization of the Colon Is More Frequent and Higher in Inflammatory or Tumorous Bowel Diseases

Colorectal adenomas represent early precursors of colorectal carcinomas, and a significant association was found between the presence of *F. n.* and colorectal adenomas ($p = 0.01$). Patients with multiple findings of *F. n.* showed a 3.5-fold increase in the occurrence of adenomas (McCoy et al. 2013). The amounts and prevalence of *Fusobacterium nucleatum* were significantly higher in adenomas compared to the control group without adenomas, with these bacteria being localized in the mucus layer above the epithelium and in the crypts of the colon (McCoy et al. 2013). A positive correlation was also confirmed between inflammatory complications in adenomas and the presence of *F. n.* (McCoy et al. 2013). In patients with adenomas and positive findings of *F. n.*, as well as in the control group without adenomas, increased concentrations of pro-inflammatory cytokines IL-6, IL-10, IL-12, IL-17, and TNF- α were observed. The statistically most significant correlation ($r = 0.443$; $p = 0.01$) was established for IL-10. At the threshold of statistical significance ($r = 0.335$; $p = 0.06$), values were reported for TNF- α (McCoy et al. 2013).

F. nucleatum does Not Colonize Intestines With a Complex and Balanced Microbiota

Most current research states that the intestinal commensal microbiota has a significant and detectable effect on digestive health, function, and the overall health status of its host. The gut microbiome consists of more than 1,000 bacterial species, predominantly localized in the large intestine, and more than 100 trillion intestinal microbial cells (Artemev et al. 2022). However, disruption of the intestinal microbiota in the sense of colonization of the gastrointestinal tract

by various bacterial pathogens—i.e., bacterial dysbiosis—can be associated with intestinal neoplasms and carcinomas. In addition to intestinal microbial or pathological dysbiosis as a risk factor, other risk factors include advanced age, high BMI, consumption of red meat, alcohol intake, genetic predispositions, and the presence of chronic inflammatory changes (Garvey, 2024; Yang & Pei, 2006). Intestinal dysbiosis, or the disturbance of the natural composition of gut bacteria, represents a serious pathological condition in which the intestines lose beneficial and protective bacteria, which are replaced by dysbiotic, carcinogenic bacteria. These may induce several malignant tumorigenic mechanisms such as cell proliferation, disruption of natural apoptosis, and increased neoangiogenesis (Artemev et al. 2022; Jahani-Sherafat et al. 2018; Zackular et al. 2013).

Some studies report the presence of dysbiosis and excessive intake of processed foods as risk factors for the development of colorectal carcinomas (Hibberd et al. 2017). Current findings suggest that the emergence of intestinal dysbiosis may provide a platform for studying the onset of colorectal carcinoma in conjunction with individual and environmental risk factors (Sobhani et al. 2013). The role of healthy intestinal microbiota can be summarized as a functioning protective mechanism, guarding against colonization by pathogenic microorganisms. However, as in all microenvironments, disruption of the intestinal microbiota may lead to the onset and occurrence of malignant neoplastic tumors. There are sufficient examples in the human body where bacteria contribute to, or even trigger, the formation of tumors within and beyond the digestive tract. Gastric cancer and cervical cancer represent microenvironments that are constantly exposed to microorganisms (Li et al. 2021). To understand the role of intestinal microbes in the etiopathogenesis

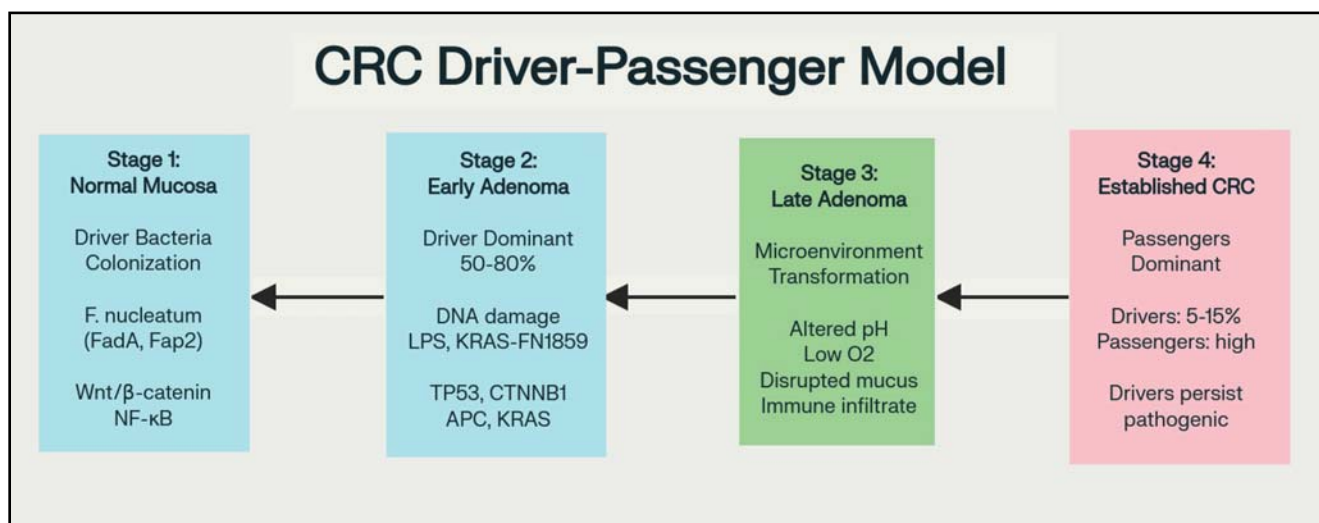


Fig. 1. CRC Driver-Passenger Model: Four-stage CRC progression showing temporal changes in bacterial composition, virulence mechanisms, and microenvironmental factors. *F. nucleatum* prevalence decreases (50-80% to 5-15%) but maintains pathogenic activity through oncogenic virulence factors. Horizontal timeline showing 4 stages with decreasing driver prevalence (50-80% → 5-15%) but persistent pathogenic activity.

of colorectal carcinoma, a model referred to as the "driver-passenger" model has been developed, which will be presented in the following text (Tjalsma *et al.* 2012).

The "Driver-Passenger" Model and the Role of Gut Bacteria in the Etiopathogenesis of Colorectal Carcinoma

The driver-passenger model distinguishes **driver bacteria** (directly initiate transformation via DNA damage, oncogenic pathways, virulence factors) from **passenger bacteria** (colonize established tumor post-transformation). *F. nucleatum* is primary driver: 50-80% tumor prevalence, FadA/Fap2/Fn-Dps virulence factors, Wnt/ β -catenin and NF- κ B activation (Tjalsma *et al.* 2012) (Fig 1).

The "driver-passenger" model presents a sequence or cascade of pathological changes consisting of irreversible mutations in stem cells located at the base of the main crypts of intestinal epithelial cells. These cells cease to undergo apoptosis and are capable of accumulating further serious mutations, such as TP53, CTNNB1, APC, and **KRAS** (52% of CRC cases). In patients predisposed to the development of CRC, the intestinal mucosa is colonized by bacteria with tumorigenic potential. In this model of "bacterial drivers-passengers," based on the adenoma-carcinoma sequence, the "driver bacteria" participate in causing DNA damage in the intestinal epithelium. Once tumorigenesis begins, microenvironment changes: altered pH, \downarrow O₂, disrupted mucus, immune recruitment, altered nutrients \rightarrow passenger colonization. *F. nucleatum* prevalence \downarrow 50-80% \rightarrow 5-15%, BUT pathogenic activity persists via constitutive FadA/Fap2/Fn-Dps expression promoting tumor growth and chemoresistance (Tjalsma *et al.* 2012; Zepeda-Rivera *et al.* 2024). Within the framework of the "driver-passenger" model, *Fusobacterium nucleatum* is cited in the first position as the species most frequently localized in CRC (Flanagan *et al.* 2014; Yamaoka *et al.* 2018; Tjalsma *et al.* 2012; Fearon & Vogelstein, 1990).

Elements and Mechanisms of Colorectal Carcinogenesis Mediated by *Fusobacterium nucleatum*

At the outset of this topic, it is necessary to emphasize that the precise causal associations between *F. nucleatum* and colorectal carcinoma are not yet fully known or established. However, numerous important studies have long presented evidence mapping various virulence factors that have advanced *F. nucleatum* to one of the leading pathogenic bacteria associated with CRC. *F. nucleatum* possesses the following important virulence factors:

1. FadA Adhesin: Primary Invasion and Oncogenic Signaling

FadA adhesin is a major factor of adhesion and invasion, actively stimulating both inflammatory and oncogenic responses of the host organism and intestinal tissue. FadA binds to cadherins of host cells and

promotes the invasion of *F. n.* into epithelial and endothelial cells (Rezasoltani *et al.* 2025; Han *et al.* 2005). FadA specifically binds to E-cadherin at an 11-amino acid region, and the peptide produced from this binding site induces the growth of CRC cells as well as inflammatory and oncogenic responses.

Research indicates that the stimulation of CRC cells through *F. n.* is strongly dependent on FadA activity, which represents a key finding (Rubinstein *et al.* 2013). In patients with adenomas and adenocarcinomas, the level of FadA genes was 10- to 100-fold higher compared to patients in the control group without adenomas (Rubinstein *et al.* 2013).

The binding of FadA adhesin to E-cadherin on the surface of CRC cells activates the canonical Wnt pathway and the β -catenin pathway (Rezasoltani *et al.* 2025). This triggers activation of the NF- κ B pathway, which enhances the virulence of lipopolysaccharide endotoxin and TLR-4 on the surface of CRC cells, leading to increased expression of the carcinogenic miR-21 (Chen *et al.* 2022; Yang *et al.* 2017). The FadA-E-cadherin interaction thus activates the β -catenin pathway, which stimulates oncogenesis and regulates colorectal carcinogenesis (Rezasoltani *et al.* 2025; Rubinstein *et al.* 2013).

An important role in the development of colorectal carcinoma is played by the Wnt/ β -catenin pathway, which is largely regulated and influenced through Annexin A1 (Rubinstein *et al.* 2019) and Annexin A2. Annexin A2 (ANXA2) is implicated in the development of various types of cancer, as it governs essential cellular processes such as invasion, migration, proliferation, and angiogenesis (Rezasoltani *et al.* 2025; Sharma, 2019).

When examining the dominant virulence factor of *F. n.*, FadA, through the expression of FadA, Annexin 2, has-let-7a-2, and LINC00460 in 30 samples from CRC patients (compared with control samples from healthy patients and 30 samples from patients with adenomas), the following results were obtained: a statistically significant correlation was identified between FadA and LINC00460 ($r_s = 0.9311$, $p < 0.0001$). Furthermore, it was found that Annexin A2 influences CRC progression through the Hippo and JAK-STAT signaling pathways. The results of this study indicate that FadA stimulates CRC progression by upregulating LINC00460, which in turn increases ANXA2 expression via the ceRNA network (Rezasoltani *et al.* 2025).

2. Fap2 Adhesin: Immune Evasion and Tumor Binding

Fap2 is a lectin located in the outer membranes of fusobacteria that mediates their binding to CRC tumors and their subsequent invasion. In patients with advanced stages of CRC, high concentrations of Fap2 from fusobacteria, as well as Fic gene families, were identified, both of which are associated with the occurrence of CRC. Fusobacteria with Fap2+ status harbor and contain high levels of Fic genes in microbial stool

samples when compared with other bacterial species linked to cancer and with the human gut microbiome (Nakatsu et al. 2025).

Furthermore, specific Fic families were identified in colon adenocarcinoma cell lines; these were present in the genomes of *Fusobacterium nucleatum animalis* strains containing Fap2, with Fic proteins being synthesized directly within *F. n. animalis* (Nakatsu et al. 2025). Fap2, as a galactose-binding adhesin protein, can attach to TIGIT receptors on the surface of natural killer (NK) cells, thereby preventing them from destroying tumor cells. On the surface of CRC cancer cells, the Gal-GalNAc receptor is present, to which *F. n.* can bind, inducing the production of proinflammatory cytokines and promoting tumor cell proliferation (Ranjbar et al. 2021; Ganesan et al. 2019).

3. *Fn-Dps: Iron Competition and Cellular Migration*

Fn-Dps is a newly discovered virulence factor of *F. nucleatum*, belonging to the group of bacterial ferritins that protect its DNA against oxidative stress. In contact with the host, it destroys erythrocytes in competition for iron acquisition. Fn-Dps enables the survival of *F. n.* in macrophages by increasing the expression of chemokines CCL2/CCL7.

Through epithelial–mesenchymal structures, it supports the transition or migration of CRC cells, which is mediated by chemokine CCL2/CCL7 (Wu et al. 2023). This mechanism represents a crucial pathway through which *F. nucleatum* promotes metastatic potential in colorectal cancer.

4. *MORN₂: Cellular Invasion Enhancement*

MORN₂, in cooperation with FadA proteins, plays an important role in the cellular invasion of *F. n.* (Ranjbar et al. 2021). MORN2 are proteinaceous substances that, via a signal sequence, allow the translocation of small peptides from the outer cell envelope of *F. n.* into the periplasmic space. The production of virulence factors by *F. n.* directly and indirectly correlates with various mechanisms of carcinogenesis mediated and stimulated by *F. n.*

5. *Lipopolysaccharide (LPS) Toxin: Inflammatory Cascade Activation*

Lipopolysaccharide toxin (LPS) activation follows the binding of FadA to E-cadherin, the Wnt/ β -catenin signaling pathway is activated (Rezasoltani et al. 2025; Rubinstein et al. 2013), which in turn stimulates NF- κ B and increases the virulence of LPS and TLR4 (Yang et al. 2017).

This further activates the enzyme PAK-1, which controls phenotypic signaling and gene expression, subsequently regulating invasion, directional cell migration, growth, and progression of the cell cycle—processes that influence angiogenesis and metastasis, thereby playing a significant role in the initiation and progression of human cancer (Radu et al. 2014).

PAK-1 triggers phosphorylation at Ser675 and stimulates the regulation of the proto-oncogene MYC, which controls transcription factors and CCND (Ou et al. 2022). The LPS toxin, via the TLR4/MYD88 receptors, induces microRNA-21, which—through Wnt/ β -catenin signaling—promotes tumor growth (Wu et al. 2022).

Fusobacterium nucleatum and Its Genetic Equipment Enabling Carcinogenesis Mediation

Patients with CRC harbor a rich representation of *F. n.* in tumors of the distal gastrointestinal tract (Mima et al. 2016; Kostic et al. 2012). In the evaluation of genetic factors facilitating *F. n.* colonization, 80 oral strains from healthy individuals and 55 cancer strains from 51 individuals with CRC were analyzed, with a total of 483 genetic factors identified from CRC patient samples. It was found that the predominantly occurring subspecies in CRC patients was *F. n. animalis*; however, genomic investigations revealed that this subspecies contains two distinct clades, designated FnaC1 and FnaC2, with FnaC2 being dominant in tumor structures. In analyses, 195 genetic factors belonging to FnaC2 were identified, associated with gastrointestinal colonization and CRC tumors. In tumor samples from 116 patients, FnaC2 was highly represented. The results of this important study revealed that the specific FnaC2 drives and causes the enormous prevalence of this clade in CRC, and the study also provides information on the genetic causes of pathological adaptation to the tumor tissue niche (Zepeda-Rivera et al. 2024). The development and progression of CRC may also involve certain oncogenic mutations from the RAS family, particularly its isoform KRAS, whose correlation with CRC is reported in up to 52% of cases (Dewan et al. 2025; Uniyal et al. 2025; Cox et al. 2014). KRAS, as the homolog of the Kirsten rat sarcoma viral oncogene, is the most well-known oncogene associated with many types of highly malignant cancers, such as NSCLC, PDAC, and CRC, with targeted KRAS therapy showing initial success in treating fatal cancers (Huang et al. 2021). *F. nucleatum* is capable of enhancing and stimulating oncogenic cascades mediated by KRAS. In another study, oncogenic pathway mutations of KRAS were present in up to 60% of CRC patients (Dewan et al. 2025). Specific findings of the oncogenic action of *F. n.* are presented by its detection and colonization of CRC tumors, which correlate with the KRAS p.G12D mutation. The direct tumorigenic effect lies in the interaction between a *Fusobacterium nucleatum* protein, named FN1859, and the nuclear helicase DHX15 of the cancer cell (Dewan et al. 2025; Huang et al. 2021; Kostic et al. 2012). These results can be considered important insights into the direct carcinogenic role of *F. n.* in the etiopathogenesis of CRC, providing arguments and motivation for the elimination of *F. n.* from the organism. An important signaling pathway responsible for CRC is chromosomal instability (CIN), which is present in up to 70% of CRC cases (Dewan et al. 2025;

Duta-Ion et al. 2024). CIN involves the absence of the APC gene, inducing Wnt signaling, inactivation of the TP53 gene, activation of KRAS, and disruption of PI3K and RAS/MAPK signaling (Dewan et al. 2025; Colombo et al. 2025). Another molecular signaling pathway in CRC lesions is the methylation of CpG islands, since the CpG island methylator phenotype (CIMP) is one of the possible routes leading to CRC lesions. Its hypermethylation in promoter regions, together with BRAF gene mutation, results in high MSI values (Nazemalhosseini Mojarad et al. 2013; Colombo et al. 2025). Significant virulence factors of *F. n.* also include its multiple immunosuppressive properties affecting macrophages, T cells, and various mechanisms of cellular immunity (Guevarra et al. 2018; Chen et al. 2018).

Treatment and therapeutic strategies

The findings from numerous studies allow us to state that *Fusobacterium nucleatum* represents the most frequent and most widely reported bacterial pathogen in the tumorigenesis of CRC lesions (Chen et al. 2022; Guo et al. 2017; Copenhagen-Glazer et al. 2015; Han, 2015; Krieger et al. 2024; Kitamoto et al. 2020; Dewhirst, 2016; DiRienzo, 2014; Kwong et al. 2018; Knippel & Sears, 2021; Boleij & Tjalsma, 2013; Strauss et al. 2011; Tahara et al. 2014; Warren et al. 2013; Tjalsma et al. 2012; Dekker et al. 2019; Ou et al. 2022; Amitay et al. 2017; Flanagan et al. 2014; Yamaoka et al. 2018; McCoy et al. 2013; Artemev et al. 2022; Garvey, 2024; Yang & Pei, 2006; Jahani-Sherafat et al. 2018; Zackular et al. 2013; Hibberd et al. 2017; Sobhani et al. 2013; Li et al. 2021). In cooperation with other risk factors, it contributes to cancer development in the distal colon and rectum, and its presence is closely correlated with progression, chemoresistance, and the immunosuppressive microenvironment of CRC (Repass et al. 2016; Manson McGuire et al. 2014; Borozan et al. 2022; Helmink et al. 2019). Considering its effects in promoting carcinogenesis, the elimination of *F. n.* is regarded as a promising strategy to improve therapeutic outcomes in colorectal cancer.

Strategic therapeutic approaches can be divided into three main directions:

- A. Elimination of *F. n.* using antibiotics**
- B. Elimination of *F. n.* from the GIT without the use of antibiotics**
- C. Elimination of *F. n.* from primary sources in oral cavity periodontitis**

A. Elimination of Fusobacterium nucleatum using antibiotics

Several bacterial species colonize not only the environment within and around CRC tumors, but through biofilms they can also colonize the direct surface of CRC lesions. The issue of biofilm formation on the surface of CRC lesions has been addressed by multiple studies investigating the aggregation and colonization by bacterial strains and species relevant to CRC

occurrence. Coaggregation abilities of *F. n.* have been identified between three strains of *F. n. animalis* and another 47 bacterial taxa associated with CRC (Robinson & Allen-Vercoe, 2023). From many environments and various structures, the mechanisms and reasons why bacteria cluster into biofilms are well studied and documented. Biofilms provide protection against extreme effects of the surrounding environment as well as against the action of antibiotics. The structure and organization of biofilms have been thoroughly investigated in the oral cavity, where *F. n.* regularly participates in subgingival biofilms covering the surfaces of periodontal pockets. A significant property of bacteria organized in biofilms is their high resistance to antibiotics. Their elimination in oral biofilms requires concentrations several hundred times higher compared to their planktonic occurrence (Sliepen et al. 2010; Wright et al. 1997). The strategy of eliminating *F. n.* and other anaerobic bacteria in the oral cavity has long been established and employed in a therapeutic approach where biofilms are first mechanically removed, followed by the application of local antibacterial agents and systemic antibiotic administration. The latter, however, does not penetrate oral biofilms and is bactericidal only at concentrations that are practically unattainable. At this point, the question arises whether the situation of *F. n.* localized within intestinal biofilms is not similar or even analogous to that observed in oral biofilms.

The intestinal microbial biofilm forms on the inner mucosal layer of the colon and consists of diverse microbial communities. The highest oncogenic potential is exhibited by the anaerobic bacteria *Fusobacterium nucleatum*, *Escherichia coli*, and enterotoxigenic *Bacteroides fragilis* (Chew et al. 2020). The formation of an intestinal polymicrobial biofilm requires the development of adhesive matrices and extracellular polymer. The intestinal patobiome serves as a source of numerous pro-inflammatory responses with pronounced pro-oncogenic properties (Li et al. 2017; Dejea et al. 2014).

Disruption of intestinal homeostasis through pathological biofilm formation is a key step in inflammation, leading to a cascade of changes that drive colorectal carcinogenesis (Kushwaha et al. 2025). The formation of invasive types of intestinal biofilms containing *F. n.* has been associated with their pathogenicity. In a cohort of 116 patients, biofilms were visualized on tumors in 68.8% of cases, while in 79.0% of patients with tumor-associated biofilms, *F. n.*-containing biofilms were also detected on distant normal tissue (Queen et al. 2025). The creation of intestinal polymicrobial biofilms contributes to reduced sensitivity of other intestinal pathogens to metronidazole and vancomycin (Rahmoun et al. 2021). The formation of bacterial biofilms with a high prevalence of *F. n.* is typical and frequent in the distal colon, accounting for up to 89% of their detection (Dejea & Sears, 2016; Liu

et al. 2024). The establishment of bacterial biofilms within the colonic mucosa includes species capable of invasive penetration into mucus with a substantial presence of *F. nucleatum* (Yu et al. 2017). For successful treatment of colorectal cancer, it is essential to maximally eliminate *F. n.* from intratumoral sites, colonic mucosa, and colorectal biofilms.

The most effective antibiotic for the elimination of *Fusobacterium nucleatum* from the human body is metronidazole, which is commonly used and recommended for its oral localization in the treatment of destructive periodontal diseases (Dabija-Wolter et al. 2018; Kapoor et al. 2012). As a first-line drug, it is also employed in gynecology for the treatment of bacterial vaginosis and trichomoniasis (Sobel & Sobel, 2015). To evaluate the effect of metronidazole in the gastrointestinal tract of CRC patients, several studies have been conducted. Some studies have shown that extensive infection of CRC tumors by *F. n.* is very common, and these patients are more susceptible to recurrences (Castellarin et al. 2012; Yu et al. 2017). Moreover, in an animal model, it was found that metronidazole could reduce the prevalence and burden of *F. n.* and inhibit the growth and proliferation of tumor cells (Yu et al. 2017).

Some renowned institutions have begun implementing long-term administration of metronidazole in various time and dosage regimens in an effort to avoid resistance to the drug. The most common complication during long-term use of metronidazole for more than 4 weeks, at a cumulative dose higher than 42 grams, was peripheral neuropathy in 17.9% of patients (Gao et al. 2023; DeDosso et al. 2025). Based on studies performed on minimal doses of metronidazole (Gao et al. 2023), a randomized, unicentric, prospective, double-blind study with a relatively large sample of 300 patients (150 experimental group + 150 control group) is currently being conducted. It examines the effect of long-term administration of metronidazole on reducing the incidence of postoperative liver metastases in CRC patients up to 5 years after surgery. Another ongoing pilot study, conducted on 40 patients in the preoperative preparation of CRC tumors, aimed to determine the efficacy of metronidazole in reducing the presence of *F. nucleatum* in intraoperatively collected CRC tissue samples. For this purpose, patients took metronidazole for 10 days (500 mg \times 3/24 h) before resection surgery. Before metronidazole medication, diagnostic biopsies were taken, which will be evaluated using PCR and compared with biopsies from resection specimens. The secondary objectives of the study included investigating the effect of metronidazole administration on the extent of immune cell infiltration and tumor cell autophagy in CRC, the impact of metronidazole on the intestinal microbiome, and the identification of metabolic changes in the gut after its application (DeDosso et al. 2025). The results of these well-designed studies will soon provide answers and

establish fundamental knowledge for the potential use of metronidazole in CRC treatment.

Associations between colorectal cancer (CRC) of the colon and the use of broad-spectrum antibiotics are primarily known for their negative and pathological effects on CRC development. The increased risk of CRC associated with repeated use of broad spectrum antibiotics has been confirmed by several large scale studies. In a population-based study from Sweden, 40,545 cases of CRC were examined, extracted from national registries, along with 202,720 patients from control groups. A strong association was found between antibiotic use and a higher risk of colon cancer, as well as an inverse association with rectal cancer in women (Lu et al. 2022). A large meta-analytic study summarized the results of 10 significant and authoritative studies, which together included 4.1 million subjects with 73,550 cases of CRC. The pooled risk of developing CRC in patients who had ever used antibiotics was ES = 1.17, 95% CI 1.05–1.30. Among patients who used broad-spectrum antibiotics, the pooled risk values were even higher, at ES = 1.70, 95% CI 1.26–2.30. In contrast, the use of narrow-spectrum antibiotics showed weaker associations, with ES = 1.11, 95% CI 0.93–1.32 (Simin et al. 2020). In a cross-sectional study, 7,903 patients with CRC were compared with a control group of 30,418 healthy subjects. Within this cohort, there was a subgroup of 445 patients with early onset CRC under 50 years of age. In comparison with the control group, their adjusted odds ratio (OR_{adj}) was 1.49 (95% CI 1.07–2.07, $p = 0.018$). In the subgroup older than 50 years, the adjusted OR was 1.17 (95% CI 1.01–1.18, $p = 0.029$) (McDowell et al. 2022). The results of these studies conclude that systemic use of antibiotics is associated with an increased incidence of colorectal cancer (Simin et al. 2020; McDowell et al. 2022).

The most frequently cited cause linking antibiotic use with CRC is the alteration of microbial balance in the intestine, leading to the overgrowth of pathogens associated with CRC such as *Fusobacterium nucleatum*, *Clostridium difficile*, *Escherichia coli*, and *Bacteroides fragilis*, followed by the development of intestinal dysbiosis or a patobiome (Boursi et al. 2015; Ferrer et al. 2017; Zhang et al. 2019; Greer & O'Keefe, 2011). Another pathological consequence of frequent and excessive antibiotic use is the disruption of fundamental immunological responses and the dysregulation of host immunity mediated by the gut microbiome (Erdman & Poutahidis, 2015; Flemer et al. 2017). Another possible cause and consequence of the overgrowth of pathogenic bacteria such as *Fusobacterium nucleatum*, *Clostridium difficile*, *Escherichia coli*, and *Bacteroides fragilis* is the increased risk of CRC through alterations in pro-carcinogenic mechanisms that stimulate the initiation and growth of cancer cells. These prooncogenic mechanisms include disturbances in apoptosis and cell differentiation, changes and impairments in cell proliferation, and the production of DNA damaging toxins (Simin et al.

2020; Minot & Willis, 2019; Arthur *et al.* 2012; Gagnière *et al.* 2016). Many studies addressing the issue of antibiotic use, as well as metaanalyses, have so far failed to establish a precise causal relationship between antibiotic use and CRC (Simin *et al.* 2020; Viljoen *et al.* 2015; Zepeda-Rivera *et al.* 2024). However, the conclusions of several investigations suggest that broad spectrum antibiotics in particular suppress the physiological gut microbiota, thereby facilitating the adhesion and colonization of the intestinal environment by pathogenic bacteria. For this reason, antibiotics are not considered suitable for the elimination of microbial pathogens that are suspected to be associated with CRC. The issue of long-term administration of low doses of selected antibiotics remains unresolved, as ongoing long-term studies are currently being conducted, with their results and conclusions still awaited (Gao *et al.* 2023; DeDosso *et al.* 2025; DeDosso *et al.* 2025).

B. Elimination of Fusobacterium nucleatum without the use of antibiotics

There is substantial evidence regarding the pro-carcinogenic effects of *F. nucleatum* in the intestinal environment, as outlined in the preceding text. Patients with CRC tumors and high concentrations of *F. nucleatum* show poorer survival outcomes, and *F. nucleatum* promotes disease progression (Mima *et al.* 2016; LaCourse *et al.* 2022). Modulation and regulation of *F. nucleatum* may therefore have a beneficial influence on the course of colorectal cancer (LaCourse *et al.* 2022). The use of antibiotics in the treatment of bacterial diseases carries with it harmful effects on the gut microbiota. Due to the adverse and damaging impact of antibiotics on the intestinal microbiota, in the treatment of CRC and in reducing the abundance of *F. nucleatum* and other pro-carcinogenic bacteria in the gut, we are compelled to search for and utilize bioactive preparations and substances from new sources, as well as novel therapeutic antimicrobial strategies and compounds. These have been divided into eight groups (Liu *et al.* 2024).

The first group consists of antibacterial agents derived from natural sources. These alternative substances are continuously being investigated and can be classified into several groups, chemically including flavonoids, polyphenols, alkaloids, steroids, saponins, quinones, and terpenes, obtained primarily from sources such as turmeric (Liu *et al.* 2024; Guglielmi *et al.* 2020). Flavonoids, curcumins, and curcuminoids from turmeric and other plants inhibit DNA replication, disrupt cell membranes, restrict microbial motility, and limit nucleic acid production. They are present in extracts and propolis formulations (Kapaonik propolis, Oxapampa propolis, turmeric rhizome, *Curcuma xanthorrhiza*) (Liu *et al.* 2024; Guglielmi *et al.* 2020; Millones Gómez *et al.* 2021). Other natural substances include various polyphenolic compounds derived from extracts of young apples, blueberries, and cranberries

(Liu *et al.* 2024; Pellerin *et al.* 2021). Extracts from American ginseng, cinnamon fruits, aloe, and pomegranate also exhibit antibacterial properties.

The second group of organic antibacterial agents includes synthesized cationic antimicrobial peptides (AMPs), which bind to anionic components of the cell membranes of Gram-negative bacteria producing LPS, where they form pores leading to envelope destruction (Liu *et al.* 2024; Shen *et al.* 2018). Representative AMP substances include L-lysine, Nal-P-113, the rice-derived protein Amyl-1-18, azurin, and IL-37, which influence the growth, maturation, and pathogenicity of *F. nucleatum* through various mechanisms (Liu *et al.* 2024; Shen *et al.* 2018). Photosensitizing oxidative substances release a range of potent oxidants upon exposure to low-intensity light, which are capable of killing and eliminating *F. nucleatum*; however, the penetration of the required light into deep tissue layers in CRC has not yet been resolved (Kunz *et al.* 2019).

The third group of antibacterial agents consists of inorganic compounds. The antibacterial effect of heavy metals has long been known and utilized. Current technologies allow their processing into nanomaterials, which exert antibacterial properties even at lower doses by disrupting the cell membrane of *F. nucleatum*, thereby preventing biofilm formation (Liu *et al.* 2024; Beyth *et al.* 2015).

The fourth group is represented by polymers. Some polymers with positively charged surfaces are capable of interacting with the negatively charged bacterial membranes and destroying them. Within these associations of membrane disruption, the quaternary ammonium-based compound PAMAM-AZO@CPA (Q-P-A@CPA) has been investigated, demonstrating the ability to inhibit the growth of *F. nucleatum* biofilms in patients resistant to conventional drug therapy for CRC (Liu *et al.* 2021). In antibacterial strategies against *F. nucleatum*, an additional four groups of different substances have been proposed and partially applied (Liu *et al.* 2024).

C. Elimination of F. nucleatum in early gingival and periodontal lesions

The essence and objective of the third strategy for eliminating *F. nucleatum* in the human body is its early eradication from the environment of subgingival oral biofilms colonizing the surfaces within periodontal pockets. These schemes and procedures require, and will continue to require, close collaboration between dentists or periodontologists and gastroenterologists or oncologists.

In the previous text, numerous virulence factors of *F. nucleatum* and their possible role in the etiopathogenesis of CRC were mentioned. The most common source of *F. nucleatum* infection in the human body is considered to be its oral presence in various forms of periodontitis, which are characterized by the creation of an anaerobic microenvironment in the form

of periodontal pockets. Several significant studies have confirmed identical strains of *F. nucleatum* in the oral cavity and in the intestinal localization in CRC (Komiya et al. 2019). The most common route of transmission from the oral cavity to the lower parts of the gastrointestinal tract is regarded as its hematogenous spread via circulation (Kitamoto et al. 2020; Abed et al. 2020). Bacteremia caused by *F. nucleatum* is considered by some authors to be a serious etiopathogenetic symptom and a trigger for disease onset and its unfavorable prognosis (Kwong et al. 2018). Colonization and representation of *F. nucleatum* in oral biofilms changes with age and with the development of oral biofilms. For the development of anaerobic pathological bacterial microflora, **the key pathological structure is the formation of subgingival biofilms**, which represent a highly specialized ecosystem providing an enormous degree of protection and resistance to the bacteria contained within. After the early colonizers of tooth surfaces—*Streptococcus* and *Actinomyces*—colonization by the late periodontal bacterial pathogens of the so-called "red complex" (*Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*) follows. These are considered the most serious periodontal pathogens and can be detected by various outpatient techniques, such as the BANA peptide test (Malvika et al. 2021). *Fusobacterium nucleatum* significantly contributes to interspecies bacterial coaggregation and the formation of multi-species bacterial biofilms in the environment of periodontal pockets, the deepening of which significantly promotes the development of an anaerobic microenvironment. Coaggregation with early biofilm colonizers such as *Actinomyces naeslundii* and *Streptococcus cristatus* is mediated by the outer membrane adhesin Rad, encoded by the gene Fn1526, with Rad expression being regulated by signaling through the CarRs transduction system (Malvika et al. 2021; Wu et al. 2021). The Rad adhesin has been shown to mediate coadherent activity with several oral pathogens, such as *Streptococcus mutans* SpaP and *Candida albicans* (Chen et al. 2022; Chen et al. 2022; Chen et al. 2022). Among extraoral adherence properties, it is important to note its adhesion to the flagellar structures of the well-known intestinal pathogen *Clostridioides difficile*, which facilitates pathological colonization of the intestine (Chen et al. 2022; Sandhu & McBride, 2018).

F. nucleatum is a significant oral anaerobic microorganism that is classified within the orange complex according to Socransky, and it forms an adherent bridge between this group and the most pathogenic bacteria of the red complex, thereby markedly increasing its pathogenicity through enhanced formation of subgingival biofilm (Sharma et al. 2005; Rosen et al. 2008). The association of *F. nucleatum* with early forms of periodontal inflammation, such as gingivitis, has been investigated in several studies, which confirm its high prevalence even in the initial stages of periodontal disease. These conditions represent reversible

inflammatory lesions of the gingiva—gingivitis that respond well to treatment. *F. nucleatum* infection targets one of the first defensive barriers in the oral cavity, namely the gingival epithelial cells, which constitute a protective shield against the penetration of bacterial pathogens into the gingiva and other periodontal tissues. Once this epithelial barrier is breached, a defensive inflammatory reaction develops within the gingival tissue in the form of gingivitis. Bacterial gingivitis is easily detectable in clinical practice through simple examinations using indices such as BOP (Bleeding on Probing) and PBI (Papillary Bleeding Index), which identify the presence of hyperemia and vascular dilatation by demonstrating increased gingival bleeding. This assessment constitutes a basic dental examination routinely performed in every dental practice.

It has been established that *F. nucleatum* actively participates in the disruption of defense mechanisms in the oral gingival epithelium through several pathways that have been relatively well described. Gingival epithelial cells form both a chemical and physical shield against infection. They produce antimicrobial proteins, β -defensins, which prevent bacterial colonization and biofilm formation. The production of defensins in gingival epithelial cells induces mechanisms of both innate and adaptive immunity (Yin & Chung, 2011). The effect of *F. nucleatum* infection on gingival epithelial cells enhances the expression of pro-inflammatory cytokines and the activation of inflammasomes within the cells themselves. The fundamental mechanism lies in the induction and stimulation of NF- κ B translocation into the nuclei of epithelial cells, leading to the expression of cytokine genes. Furthermore, the NLRP3 inflammasome is activated even in the absence of exogenous DAMPs, which triggers caspase-1 activation and induces the secretion of mature IL-1 β , along with the release of DAMP components such as apoptosis-associated speck-like protein and high-mobility group box 1 protein, both of which mediate inflammation progression (Bui et al. 2016). All these aggressive pro-inflammatory responses activate a cascade of additional pro-inflammatory substances, further driving the progression of inflammation. Based on these findings, it is evident that *F. nucleatum* is present already in the early stages of gingivitis, which can be routinely diagnosed and treated. Nevertheless, in acute gingivitis, invasion of *F. nucleatum* into the periodontal tissues themselves is not typically expected, which constitutes a fundamental prerequisite for its potential hematogenous dissemination into the gastrointestinal tract.

At this point, we may ask the question: where is the threshold or turning point in the pathological progression of periodontitis at which bacterial invasion of periodontal tissues occurs? Despite the fact that sufficient evidence is not currently available to either support or exclude bacterial invasion as a key step in the etiopathogenesis of periodontal disease, further research is required in this area (Mendes et al. 2015). However,

once *Fusobacterium nucleatum* and other periodontal pathogens invade gingival or periodontal tissues, hematogenous dissemination to tissue and organ structures within the body, including the colon, becomes possible. The fundamental pathological structure of periodontitis is the periodontal pocket, the walls of which are covered with a continuous layer of mature subgingival biofilm. The inner surface of the periodontal pocket on the gingival side is lined with ulcerated epithelium that has lost its immune protective functions, thereby allowing direct bacterial invasion into the tissue. Once invaded, bacteria penetrate into the surrounding connective tissue of the periodontal apparatus and alveolar bone. Their reservoir is the omnipresent subgingival biofilm, from which they directly invade periodontal tissues through the ulcerated epithelium. These invaded bacteria are in direct contact with the vascular circulation, enabling their dissemination throughout the organism. It has been determined that 1 mg of subgingival biofilm contains 10^8 to 10^9 bacteria, enormous amounts of pro-inflammatory mediators, and large quantities of lipopolysaccharide (LPS) toxins produced by Gram-negative bacteria (Mendes *et al.* 2015; Colombo *et al.* 2015; Dhotre *et al.* 2017). Transient bacteremia has been observed in patients with dental plaque accumulation and gingival inflammation in the form of gingivitis (Silver *et al.* 1977), during routine probing with a periodontal probe (Daly *et al.* 2001), in the course of standard home and professional oral hygiene procedures (Lofthus *et al.* 1991; Carroll & Sebor, 1980), as well as during various dental interventions (Lockhart *et al.* 2008; Kinane *et al.* 2005; Kinane *et al.* 2005). Another study revealed transient bacteremia after ultrasonic scaling in 23% of patients, after probing with a periodontal probe in 6% of patients, and following routine toothbrushing in 3% of patients (Kinane *et al.* 2005).

The aforementioned facts entitle and oblige dentists to the early elimination of the anaerobic microenvironment and to the removal of oral structures enabling adhesion, colonization, and invasion of *Fusobacterium nucleatum* in the oral cavity, where debridement, cleansing, and bactericidal procedures against *F. nucleatum* are relatively easy to implement and accessible.

RECOMMENDATIONS FOR CLINICAL PRACTICE

The results of the aforementioned studies indicate that consistent removal of biofilms and treatment of initial stages of gingival and periodontal inflammation, in various clinical forms of gingivitis and early stages of periodontitis, represent fundamental preventive and prophylactic approaches for the eradication of *Fusobacterium nucleatum* from oral and periodontal tissues, thereby preventing its potential hematogenous dissemination into distal parts of the colon. However,

the duties of dentists and periodontists in patients with periodontitis should also include active anamnesis and screening for chronic inflammatory bowel diseases (IBD), intestinal adenomas and polyps, chronic intestinal infections, non-polypoid colonic lesions, and a familial history of colorectal cancer (CRC). In cases of simultaneous occurrence of periodontitis and any of the aforementioned diseases of the distal colon, eradication of *F. nucleatum* from the oral environment should be initiated by all available methods, including mechanical and chemical debridement in the form of scaling, deep scaling, curettage of soft gingival tissues, followed by local treatment with chlorhexidine and other antibacterial agents. The limitations of systemic application of metronidazole, as the most effective antibiotic against *F. nucleatum*, have been discussed in the preceding text. Sufficient evidence, however, exists regarding its local elimination in periodontal pockets through the application of gels and fibers containing metronidazole, chlorhexidine, and tetracycline, which are commercially produced and applied directly into the periodontal pocket environment. One of the requirements for their local use is the prevention of their transport into the gastrointestinal tract (Gandhi *et al.* 2025; Sato *et al.* 2008; Leiknes *et al.* 2007).

The aim of periodontal therapy is to **eliminate adherent and colonizing periodontal pathogens before their invasion into the gingival epithelium and deeper periodontal tissues, from where the potential for hematogenous dissemination is significantly increased.** The attack on epithelial cells is a typical feature of invasive bacterial pathogens. *Fusobacterium nucleatum* and *Prevotella intermedia* represent two bacteria of the orange complex with highly developed invasive capacities, capable of actively targeting gingival fibroblasts and endothelial cells, with documented invasive cooperation with other oral pathogens (Kornman, 2008; Dabija-Wolter *et al.* 2009; Ji *et al.* 2010). Subgingival accumulation of biofilms involving *F. nucleatum* produces proteases with a strong invasive ability into gingival epithelial and deeper connective tissues, which attracts large numbers of pro-inflammatory cells to the sites of invasion. Disruption of innate immunity and imbalance between Th1 and Th2 cells compromise the defensive capacity of the gingiva, thereby enabling intracellular survival of periodontal pathogens, which are additionally replenished from subgingival biofilms as their permanent reservoir (Manor *et al.* 1984). Persistence of bacteria within the tissue results in further impairment of immune systems, potentially leading to hematogenous dissemination throughout the body.

CONCLUSION

A probing depth of up to 4 mm is generally considered the critical threshold for periodontal pockets, while multiple periodontal pockets of ≥ 5 mm are typically

regarded as advanced forms of periodontitis with bacterial invasion into periodontal tissues (Loesche & Grossman, 2001; Shi *et al.* 2018). The goal of the proposed cooperation between dentists and gastroenterologists in at-risk patients such as those with IBD, colorectal adenomas and polyps, chronic intestinal infections, non-polypoid colorectal lesions, and a family history of CRC is the early detection, diagnosis, and treatment of periodontitis with pocket depths around 4 mm, in which massive invasion into periodontal structures is not expected. In such cases, debridement, decontamination, and antibacterial methods aimed at eliminating periodontopathogenic bacteria show a favorable prognosis, even without systemic administration of antibiotics, which should preferably be avoided (Mombelli, 2018).

In addition to the general principles of eliminating major periodontal pathogens from the oral environment and oral structures, it is necessary and highly desirable that all dentists and periodontists engaged in the treatment of gingivitis and periodontitis conduct targeted anamnesis. In close collaboration with specialists in gastroenterology, cardiology and vascular medicine, urology, gynecology and obstetrics, diabetology, orthopedics, and neurology, they should identify and record potential systemic diseases associated with periodontitis. In patients with simultaneous occurrence of periodontitis and any of these periodontitis-associated conditions, it is essential to ensure eradication of the most virulent periodontal pathogens from the environment of periodontal pockets and periodontal tissues. This significantly reduces the likelihood of hematogenous dissemination of these bacteria into non-oral tissues and systems. Periodontal pockets with a depth of up to 4 mm are considered to allow relatively uncomplicated eradication of bacteria.

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