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TINJAUAN PUSTAKA

The Role of Microbiota and Early Detection in Colorectal Cancer Through Fecal Screening

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Abstrak

Tujuan: Tinjauan pustaka ini mengeksplorasi hubungan antara mikrobiota usus dan kanker kolorektal, dengan fokus terhadap deteksi dini melalui pemeriksaan feses. **Metode:** Peneliti melakukan tinjauan literatur komprehensif dengan menggunakan Google Scholar dan PubMed, dengan kata kunci utama termasuk "Mikrobiota," "Kanker kolorektal," dan "Pemeriksaan feses." **Hasil:** Mikrobiota usus memainkan peran penting dalam perkembangan kanker kolorektal, melibatkan strain bakteri tertentu seperti *Fusobacterium nucleatum, Escherichia coli, Bacteroides fragilis, Streptococcus bovis, Enterococcus faecalis*, dan *Peptostreptococcus anaerobius*. Strain-strain ini berkontribusi pada kanker melalui berbagai mekanisme. Pemeriksaan mikrobiota feses, seringkali menggunakan teknik canggih seperti next-generation sequencing menunjukkan potensi untuk deteksi dini. Menggabungkan data mikrobiota feses dengan metode pemeriksaan non-invasif lainnya dapat meningkatkan sensitivitas dan efisiensi deteksi kanker kolorektal. Memahami hubungan antara mikrobiota dan kanker kolorektal sangat penting untuk mencegah kanker dan diagnosis dini. **Kesimpulan:** Hubungan kompleks antara mikrobiota dan kanker kolorektal, yang dipicu oleh strain bakteri tertentu dan inflamasi, memberikan peluang untuk deteksi dini. Pemeriksaan mikrobiota feses, yang digabungkan dengan metode non- invasif, dapat mengurangi kematian akibat kanker kolorektal, meningkatkan pencegahan dan metode diagnosis kanker kolorektal.
Kata kunci: Microbiota; Colorectal Cancer; Fecal Screening; Early Detection

Abstract

Objective: *This literature review explores the link between gut microbiota and colorectal cancer, emphasizing early detection through fecal screening.* ***Method:*** *We conducted a comprehensive literature review, using Google Scholar and PubMed, with key search terms including "Microbiota," "Colorectal cancer," and "Fecal screening."* ***Results:*** *The gut microbiota plays a vital role in colorectal cancer development, involving specific bacterial strains such as Fusobacterium nucleatum, Escherichia coli, Bacteroides fragilis, Streptococcus bovis, Enterococcus faecalis, and Peptostreptococcus anaerobius. These strains contribute to cancer through various mechanisms. Fecal microbiota screening, often employing advanced techniques like next-generation sequencing, shows promise for early detection. Combining fecal microbiota data with other non-invasive screening methods can enhance colorectal cancer detection sensitivity and efficiency. Understanding this microbiota-cancer relationship is pivotal for cancer prevention and early diagnosis.* ***Conclusion:*** *The intricate microbiota- colorectal cancer connection, driven by specific bacterial strains and inflammation, presents opportunities for early detection. Fecal microbiota screening, combined with non-invasive methods, may reduce colorectal cancer mortality, advancing oncology prevention and diagnosis.*
Keywords: *Microbiota; Colorectal Cancer; Fecal Screening; Early Detection*

INTRODUCTION

The incidence and mortality of colorectal cancer vary significantly between countries and regions worldwide.[1] In Indonesia, colorectal cancer ranks as the third leading cause of cancer- related deaths. According to the Indonesian Ministry of Health (2018), there were a total of 15,985 cases in males and 11,787 cases in females with colorectal cancer.

Environmental and genetic factors are the primary causes of colorectal cancer, inducing tumorigenesis in the epithelial cells of the colon and rectum. Among environmental risk factors, the gut microbiota is also reported to contribute to the occurrence of colorectal cancer. There is increasing evidence that the gut microbiota plays a crucial role in the initiation, development, and metastasis of colorectal cancer. Due to the association between gut microbiota and human health, it is crucial to analyze the relationship between changes in gut microbiota and the occurrence, development, and prognosis of diseases. Some studies have found alterations in the fecal microbiome of colorectal cancer patients. Therefore, fecal analysis can be used for screening the risk of colorectal cancer incidence, enabling timely treatment with favorable clinical outcomes. Effective screening biomarkers leading to substantial early detection will reduce colorectal cancer mortality. In addition to the traditional invasive endoscopic approach, several non-invasive early colorectal cancer screening methods can be employed, such as fecal immunohistochemistry tests, which have been widely used due to their impact in reducing colorectal cancer incidence and mortality. However, the sensitivity of these techniques is still debated. Hence, there is a need for an efficient, safe, affordable, and non-invasive screening tool with high sensitivity for colorectal cancer. Some studies have shown the potential to combine fecal microbiota data with fecal immunohistochemistry tests to improve colorectal cancer detection.[7] Therefore, the purpose of this literature review is to elucidate the role of microbiota in colorectal cancer and the role of early detection for screening patients with colorectal cancer through fecal screening.

METHOD

This study is based on a literature review of internationally published research articles. The research data was gathered from reputable academic sources, specifically from Google Scholar and PubMed journal databases. The search strategy included the use of carefully selected keywords and phrases to retrieve relevant articles. The primary keywords employed for the search were: “Microbiota”, “Colorectal cancer”, “Microbiota and Colorectal cancer”, “Fecal screening”, “Microbiota and Fecal screening”, “Colorectal cancer and Fecal screening”.

RESULT AND DISCUSSION

The role of the microbiota and colorectal cancer

Colorectal cancer is one of the most common malignancies in the Western world and often leads to fatalities. It is well-established that food consumption and nutrition significantly affect the risk of developing colorectal cancer. Diet can have an impact on the

host's immune response and induce inflammation. It is estimated that approximately 20% to 30% of colorectal cancer cases are associated with inflammation. Inflammatory mechanisms act as drivers of tumorigenesis (Figure 1). Dietary behaviors have a significant impact on the composition of gut microbiota, which in turn affects susceptibility to intestinal diseases. Early-life antibiotic exposure has been associated with an increased risk of colorectal adenomas at the age of 60, suggesting that dysbiotic microbiota is acquired and maintained over a longer period. The large intestine and ileocecal valve show the highest bacterial density along the digestive tract, which may indicate the crucial role of microbiota in colorectal cancer. Various studies in colorectal cancer patients and experimental evidence in animal models suggest a link between gut microbiota and the occurrence of colorectal cancer. Furthermore, specific bacterial species that can promote tumorigenesis have also been identified.[9]

The first study connecting gut microbiota with the onset of colorectal cancer was reported by Weisburger *et al*.[11] Subsequently, more and more studies have confirmed the relationship between pathogenic bacteria and colorectal cancer. For example, infection with *Streptococcus bovis*, a gram-positive coccus, has been reported as a marker for the risk of developing colorectal cancer.[2] Furthermore, Kostic *et al.,* identified a high abundance of Fusobacteria sequences in colorectal carcinoma tissues using whole-genome sequencing.[3] In another study, Enterotoxigenic *Bacteroides fragilis* and *Fusobacterium nucleatum* were identified in colorectal cancer tissues, with *Fusobacterium nucleatum* being found to be associated with high microsatellite instability.[4] In another study, *E. coli* microbiota associated with mucosa belonging to phylogroup B2 was found to be more common in colorectal cancer tissues, and it was identified to encode cyclomodulin, which is crucial for mutations in colonic epithelial cells.[5] Furthermore, Zhao *et al.,* conducted a study on fecal samples from colorectal cancer patients in China and found that *Bacteroides fragilis*, *Enterococcus*, *Escherichia/Shigella*, *Klebsiella*, *Streptococcus*, and *Peptostreptococcus* showed relatively higher quantities in colorectal cancer patients. In another study, researchers also compared fecal samples and discovered that colorectal cancer patients exhibited a variation in microbiota, with lower amounts of Clostridia and higher amounts of *Fusobacterium* and *Porphyromonas*.[6]

The microbiota in the lumen and tissues clearly differ in microbial structure. In tissue samples, beneficial microbes like *Bifidobacterium*, *Faecalibacterium*, and *Blautia* are significantly reduced, while *Fusobacterium* is higher in colorectal cancer patients. However, fecal (lumen) samples show a significantly different microbial landscape, with *Paraprevotella*, *Eubacterium*, and several other bacteria displaying higher quantities in colorectal cancer patients.[12]

Types of microbiota strains in colorectal cancer

Several reports have observed that colorectal cancer tissues are associated with the presence of several bacterial strains, namely *Fusobacterium nucleatum*, *Escherichia coli*, *Bacteroides fragilis*, *Streptococcus bovis*, *Enterococcus faecalis*,

and *Peptostreptococcus anaerobius* (Table 1).[4,7,9] Several candidate pathogenic bacteria play a crucial role in colorectal carcinogenesis by adhering to the mucosal surface. Bacterial adhesion often serves as the initial step in tumorpromotion.[7]

Fusobacterium nucleatum (Fn)

Fusobacterium nucleatum is a Gram-negative, and it is an anaerobic commensal often associated with human colorectal cancer, as observed in genome studies.[3,17] Because the abundance of Fn increases from the rectum (2.5%) to the cecum (11%), this bacterial strain is believed to be relevant at specific colon locations (such as the right-sided tumors).[18] A cohort study from Japan revealed Fn colonization in 8.6% of colorectal cancer subjects and confirmed an association with MSI.[19] In another clinical study, Fn was detected in colorectal cancer tissues in 76 (13%) out of 598 cases and showed an inverse correlation with CD3+ T cell density. These findings support the hypothesis that the disease mechanism involves the regulation of the immune response.[20] The abundance of Fn is not only associated with the promotion of colorectal cancer but also confers chemoresistance and recurrence in colorectal cancer patients by disrupting the TLR4 and MyD88 signaling. Fn has been shown to target specific microRNAs that result in the activation of the autophagy pathway, thereby altering the chemotherapy response.[21] In a study regarding the types of microbiota associated with colorectal cancer, Fn was reported to be detected in metastasis. In a xenograft model of human primary colorectal cancer in mice with Fn, antibiotic treatment was shown to reduce the Fn load, tumor cell proliferation, and its growth.[22] Therefore, from several clinical pieces of evidence collected, it can be concluded that Fn is likely to promote the occurrence of neoplasia in colorectal.[9]

In some experimental evidence, Fn has been reported to inhibit the killing of NK cells by various tumors, and this effect is mediated by human T-cell immunoglobulin and ITIM domain (TIGIT).[23] The inhibitory TIGIT receptor is found in both human NK cells and T cells, and this inhibitory effect has been demonstrated to depend on Fap2 protein. This data indicates that Fn-derived factors are capable of modulating tumor-immune evasion. Furthermore, infectionof colorectal cancer cells with Fn can increase proliferation rate, invasive activity, and the potential to induce tumor xenografts in mice.[24] Fn has been shown to modulate the tumor- immune microenvironment and the E-cadherin/β-catenin signaling pathway. Rubinstein and colleagues demonstrated that Fn, through FadA adhesion, binds to E-cadherin, thereby activating the β-catenin signaling pathway, resulting in the induction of oncogenic responsesand inflammation.[25] The gene expression of FadA in human colorectal cancer tissues shows a significantly higher amount compared to healthy controls, and inhibiting this pathway is protected from pro-oncogenic activity. Fn can increase tumor multiplicity in the adenomatous polyposis coli/multiple intestinal neoplasia ($Apc^{Min/+}$) model of intestinal tumorigenesis.[26]

Escherichia coli

Although certain strains of *E. coli* have the ability to promote intestinal inflammation and produce toxins like colibactin with oncogenic potential, the *E. coli* microbiota is categorized as

intestinal commensals.[27] Dietary patterns, especially in the Western world, influence the composition of microbes and increase susceptibility to potential invasive pathogenic *E. coli*.[28]

E. coli associated with mucosa is significantly more common in colorectal cancer tissues and correlates with tumor stage and prognosis.[14] Interestingly, pathogenic *E. coli* strains expressing colibactin are more commonly found in advanced diseases, and colonization of *E. coli* strains associated with colorectal cancer into $Apc^{Min/+}$ mice results in an increased number of polyps, indicating that certain *E. coli* strains may have the potential to promote tumorigenesis.[9] Studies using bioluminescent inflammation probes and optical fluorescence imaging also indicate a correlation between *E. coli* and pro-inflammatory infiltrates that can promote tumor growth.[29] This concept is strongly supported by previous experiments on $Il10^{-/-}$ mono-colonized mice, which demonstrated that host inflammation is crucial for the activity of *E. coli* in promoting cancer events.[30]

Disease phenotype is associated with changes in the *E. coli* gene catalog, such as the polyketide synthase island (pks) that promotes tumor formation by encoding colibactin.[30] Mammalian epithelial cell cultures exposed to *E. coli* pks-positive strains exhibit transient DNA damage responses with disruptions in DNA repair and an increased frequency of gene mutations.[31] Although the exact pathomechanism remains unclear, data from xenograft and inflammation-related tumor models provide evidence that *E. coli* expressing pks encoding genotoxins (such as colibactin) enhance tumor growth.[32] This effect is partially mediated by cellular aging induced by colibactin and may involve the production of hepatocyte growth factor, which is expressed in human colorectal cancer. Interestingly, small-molecule inhibitors of ClbP, an enzyme involved in colibactin synthesis, control colibactin production and tumor formation in vivo.[33] The role of *E. coli* in colorectal cancer is further supported by metagenomic studies in large populations of colorectal cancer patients.[16]

Bacteroides fragilis

Like *E. coli*, most experimental evidence supports the role of *Bacteroides fragilis* (Bf) in intestinal tumorigenesis. Bf makes up approximately 1%–2% of the commensal microbiota in most humans. Bf-derived toxin (BFT) causes inflammatory diarrhea and inflammation- associated tumorigenesis.[34] A study by Wu *et al.*, revealed that enterotoxigenic *Bacteroides fragilis* (ETBF), which produces the toxin BFT, induces colitis and colon tumors in $Apc^{Min/+}$ mice. This phenotype is driven by Th17 cell-mediated inflammation, as neutralizing IL-17 and IL-23 reduces inflammation and tumor formation. The gene expression of Bf toxin has been detected more frequently in colorectal cancer subjects compared to controls, which also correlates with tumor prognosis.[35] ETBF colonization can be completely eliminated with cefoxitin treatment, which is paralleled by a reduction in murine colon tumorigenesis and IL- 17A expression.[36] BFT meningkatkan spermine oxidase, enzim katabolik poliamina, sehingga menghasilkan spesies oksigen reaktif dan merusak DNA, yang pada akhirnya menyebarkan peradangan dan tumorigenesis.[37]

Other microbiota

Streptococcus bovis, a Gram-positive coccus, has been reported as a risk factor for colorectal cancer.[2,38] *Streptococcus bovis* is sometimes found as part of the human digestive tract flora

and shows an increase in patients with colorectal cancer. Its role in the development of colorectal carcinogenesis is likely mediated through the inflammatory pathway, which includes factors such as IL-1, COX-2, and IL-8.[7]

Several studies have reported that *Enterococcus faecalis* is significantly higher in patients with colorectal cancer compared to healthy controls.[6,39] *Enterococcus faecalis* infection induces the production of superoxide, which damages DNA in epithelial cells. Infection with *Enterococcus faecalis* in macrophages induces superoxide production. In vitro and in vivo studies suggest that *Enterococcus faecalis* can generate hydroxyl radicals, which are potent mutagens causing DNA strand breaks, point mutations, and protein-DNA cross-linking, contributing to chromosomal instability and the risk of colorectal cancer.[7]

In a study of fecal and mucosal microbiota, Yu *et al.,* found that patients with colorectal cancer had a high abundance of *Peptostreptococcus anaerobius*. *Peptostreptococcus anaerobius* is typically found in the oral and intestinal cavities. It was discovered that *P. anaerobius* promotes the development of colorectal cancer in $Apc^{Min/+}$ mice through its surface protein, putative cell wall binding repeat 2 (PCWBR2). PCWBR2 directly binds to the α2/β1 integrin receptor on intestinal epithelial cells to initiate the oncogenic PI3K-Akt signaling pathway, driving tumor cell proliferation. *Peptostreptococcus anaerobius* can induce a pro- inflammatory microenvironment to promote tumorigenesis. In $Apc^{Min/+}$ mice, *Peptostreptococcus anaerobius* has been reported to induce the expression of pro- inflammatory cytokines, which then recruit a range of immune cells to infiltrate the tumor, particularly immunosuppressive myeloid-derived suppressor cells, tumor-associated macrophages, and tumor-associated granulocytic neutrophils, ultimately enhancing tumor development.[7,40]

Fecal Microbiota Screening

In the past, gut microbiome analysis relied on isolation and culture methods, but these methods were challenging for cultivating abundant anaerobic bacteria in the gut, greatly affecting the accuracy of the analysis. In recent years, the development of next-generation sequencing (NGS), which can accurately analyze microbial components without the need for culture, has gained attention in gut microbiota research. However, it is crucial to collect appropriate gut microbiota samples for NGS. Current sampling methods to obtain specimens from feces, mucosa biopsies, luminal brushes, laser microdissection, catheter aspiration, intelligent capsules, in vivo surgery, and FISH methods all have some drawbacks (Table 2).[41]

Because of practicality, feces are generally used for gut microbiota analysis. In practice, the process begins with the automated collection of fecal samples from home using various collection devices and storage containers. Although the impact of this step in the overall process may be crucial, there have been no systematically comparative assessments of the practices that have been conducted. Additionally, it is important to note that the composition of fecal microbiota is not identical to the

composition of microbiota found in various compartments of the digestive tract.[42] To further understand these differences, the biogeography of gut microbiota has been evaluated in several studies. Even if differences exist, it has been reported that the contents of the large intestinal (colon) luminal microbiota, where reduced transit time and high nutrient availability are observed, correlate with feces in terms of species diversity and bacterial abundance.[43,44] The contents of the small intestine (ileum, jejunum, and duodenum) luminal microbiota contain fewer microbial nutrients, are exposed to bile acids and pancreatic enzymes, and have shorter transit times, resulting in reduced diversity and abundance.[45] It is clear that the gastric microbiota is significantly different, with low diversity and abundance due to extreme acidic conditions. Additionally, the microbiota associated with the outer mucosal layer of the colon is not identical to the luminal microbiota in the same compartments, in both healthy and diseased conditions. Within the mucosa itself, the inner mucus layer and crypts containing intestinal stem cells are expected to be free from bacteria. Despite these limitations, most gut microbiota studies are conducted using fecal samples, which are easily collected in a non-invasive manner and are considered to reflect the overall variation of the colon microbiota.[46]

Several studies have found changes in the fecal microbiome of patients with colorectal cancer. Therefore, feces can be used for screening the risk of colorectal cancer incidence, allowing for timely treatment with good clinical outcomes. Effective screening biomarkers that lead to early detection will substantially reduce colorectal cancer deaths. In addition to classic invasive endoscopic approaches, several non-invasive early colorectal cancer screening tools can be used, such as fecal immunochemistry tests that have been widely adopted due to their impact on reducing the incidence and mortality of colorectal cancer. However, this technique is still debated due to its relatively low sensitivity. Therefore, there is a need for efficient, safe, affordable, and non-invasive screening tools with high sensitivity for colorectal cancer. Some studies have shown the potential to combine fecal microbiota data with fecal immunochemistry tests to improve colorectal cancer detection.[7]

Collecting feces is not as straightforward as one might think, and there are several different methods that can be used. Firstly, the question that needs to be addressed is whether to collect all material associated with a single bowel movement or just a sample of feces taken from that material. This can impact the detection and quantification of bacteria, especially for taxa with low abundance.[46] For example, Wu and colleagues reported that 35% of low- abundance taxa (<0.5% of the total) detected in one aliquot of feces were not detected in thesecond aliquot taken from the same fecal specimen.[47] This can occur due to layered biostructures observed from the outside to the inner part of the stool and/or the selective association of chosen taxa with particles that may not be evenly distributed within the specimen.[46] After fecal specimens are collected, they should be promptly frozen to avoid potential degradation. Freezing directly at -80°C is considered the reference standard. In a comparative study, it was emphasized that there was

no significant difference between the 16S repertoire generated from fresh samples (DNA extracted immediately), samples quickly frozen on ice and then frozen for 7 days at -80°C before DNA extraction, or samples directly frozen at -80°C for 7 days before DNA extraction. In the same study, the authors also observed that when analyzing the microbiota composition at the genus level for *Faecalibacterium* and *Leuconostoc*, there were significant differences in fresh samples compared to the quickly frozen group.[48] Some comparative studies indicate that direct freezing at -20°C in a patient's home freezer can also be used; however, in this case, the lack of temperature monitoring canbe an issue.[46,49]

CONCLUSION

Emerging evidence suggests a crucial role of gut microbiota in the initiation, development, and metastasis of colorectal cancer, with specific bacterial strains associated with tumorigenesis and inflammation. These strains include *Fusobacterium nucleatum*, *Escherichia coli*, *Bacteroides fragilis*, *Streptococcus bovis*, *Enterococcus faecalis*, and *Peptostreptococcus anaerobius*. Fecal microbiota screening, particularly using advanced techniques like next- generation sequencing, offers potential for early colorectal cancer detection. Combining fecal microbiota data with non-invasive screening methods holds promise for improving detection sensitivity and efficiency. Understanding this complex relationship is pivotal for advancing colorectal cancer prevention and early diagnosis.

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CONFLICT OF INTEREST

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