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The overall structure of microbiota in patients with adenocarcinoma stomach and colon cancer

O. V. Movchan

Kharkiv Institute for medical Radiology and Oncology named V.G.Grigoriev NAMS of Ukraine, Kharkiv, Ukraine

Abstract

Introduction

Accumulating evidence suggests that the human bowel microbiota contributes to the etiology of colorectal cancer (CRC) and stomach cancer, not only via the pro-carcinogenic activities of specific pathogens but also via the influence of the wider microbial community, particularly its metabolome.

The aim of the study

The aim of this study was to analyzes the overall structure of microbiota in patients with adenocarcinoma of colorectal and the stomach and healthy controls.

Results

The studies suggested that colorectal cancer and stomach cancer develops from the complex interactions between inherited susceptibility and environmental factors, there a strong association between adenomatous polyps and some pathogenic bacteria are the precursors of the vast majority of colorectal cancers and stomach. Thus, the data suggested the development of intestinal dysbiosis in patients with adenocarcinoma, which was characterized by inhibiting obligate protective microflora and activation opportunistic microorganisms on the base of carbohydrates, fats, and proteins metabolism disturbances and accumulation of toxic metabolic products that may be an important pathogenetic factor of

tumor tissue activation, induction, proliferation, and metaplasia. Significant accumulation of biogenic amines (methylamine, serotonin, histamine) is a leading metabolic profile of microflora inpatient with adenocarcinoma and may have a predictive value for diagnosis, pathogenetic therapy, and determination of food nutrients role in the mechanisms of cancer formation, as well as identifying populations of cancer risk.

Conclusions

The composition of the tumor microbiome differed from that of adjacent non-neoplastic tissue. The subsite-specific alterations in the colorectal carcinoma and stomach cancer microbiota. There is a high incidence of colorectal cancer and stomach cancer associated with Streptococcus Bovis. These results suggested that the mucosa-associated microbiota is dynamically associated with colorectal carcinoma, which may provide evidence for microbiota-associated diagnostic, prognostic, preventive, and therapeutic strategies for colorectal carcinoma and stomach cancer.

Key words: microbiota; tumor tissue activation; colorectal carcinoma and stomach cancer; biogenic amines; Streptococcus Bovis.

Accumulating evidence suggests that the human bowel microbiota contributes to the etiology of colorectal cancer (CRC) and stomach cancer, not only via the pro-carcinogenic activities of specific pathogens but also via the influence of the wider microbial community, particularly its metabolome. Recent data have shown that the short-chain fatty acids acetate, propionate, and butyrate function in the suppression of inflammation and cancer, whereas other microbial metabolites, such as secondary bile acids, promote carcinogenesis. Streptococcus Bovis is a normal inhabitant in the human gastrointestinal tract that can cause bacteremia, endocarditis, and urinary infection. Although Streptococcus Bovis was the second greatest cause of infectious endocarditis, it is frequently associated with gastrointestinal lesions, especially carcinoma of the colon and stomach. The normal microflora of the human body was formed during the evolutionary world development process. The mutual adaptation of environmental bacteria and macroorganism during appropriate selection resulted in the formation of a symbiotic complex ecosystem which is a necessary condition for human existence in creating and maintaining a favorable environment for microbes - symbionts [1 - 4]. The most important functions of the bowel microflora are nutritional (microbes metabolic products are a source for epithelial cells nutrition and stimulate bowel motility); protective (provides colonization resistance, forms a biofilm that to prevent the adhesion of pathogenic microbes, provide lysozyme, organic and free fatty acids secretion, increases the rate of cell

renovation, etc.); metabolic (is involved in the metabolism of fats and undigested nutrients, cholesterol and biologically active substances synthesis); immunostimulating (induces the synthesis of immunoglobulins and immunocompetent cells) [1 - 5]. Interruption of any of these functions leads to disruption of various kinds of structural and metabolic relationships and appearance. A vast number of microbes in the gastrointestinal tract is estimated in trillions [6]. Sarcina, enterococci, lactic acid bacteria, rarely Escherichia Coli is found in the stomach and is well tolerated to acid. In minor quantities, such microbes as enterococci, streptococci, Escherichia Coli, Lactobacilli, rarely yeasts are found in the small intestine. Bowel microflora is abundant and diverse. But the qualitative composition of those microorganisms in healthy people remains more or less constant. Among the Escherichia Coli dominates, enterococci (Enterococcus Faecalis), lactose negative Escherichia Coli, Staphylococcus, Proteus, yeasts are not more than 10-15% of the total microflora [7 - 9]. Nonsporeforming and spore-forming anaerobic bacteria are lactic acid bacilli, nonpathogenic Bacteroides, Bifidobacterium, Clostridium(Clostridium Putrificus, Clostridium Perfringens, etc.). Microbial associations create a microbial landscape specific to each non-sterile body cavity. Neutral, synergistic, antagonistic are types of relationships established between dominant and subordinate microbial species. The existence of each type in the ecosystem is biologically proved since it ultimately turns out to be necessary for the providing of complex symbiotic relationships between macro-and microorganisms. Obligate bowel microflora to the greatest extent has useful physiological functions and includes Escherichia, Bacteroides, Bifidobacterium, enterococci, and lactobacilli. Facultative microflora consists of Proteus, staphylococci, clostridia and yeast [10 - 13]. For example, Bifidumbacteria producing lactic, acetic, formic, and succinic acids create an acidic environment in the bowel to prevent its colonization by pathogenic organisms. Lactobacilli during fermentation of lactic acid from such antibiotic substances as lactogen, lactocidine, acidophilic. Representatives of the normal bowel microflora inhibit the growth and proliferation of opportunistic and pathogenic microorganisms (enteropathogenic Escherichia Coli, Klebsiella, Proteus, Salmonella, Shigella, Staphylococcus Aureus, etc.) [14] of micronutrient and immune deficiency [15].

They are bacteriological which means a determination of the fecal microflora composition and biochemical which includes a rapid method of determining the proteolytic activity of fecal supernatants, high-voltage paper electrophoresis for the detection of β-aspartylglycine, β-aspartyllysine, β-alanine, 5-aminovaleric, and γ-aminobutyric acids, etc., ion chromatography for determination of biogenic amines, bile, carboxylic acids, aromatic compounds, gas-liquid chromatography for detection of fatty acids (acetic, valeric, caproic,

isobutyric, etc.) in the feces. Currently, the main and most common method of laboratory diagnosis of dysbiosis is a bacteriological examination of patients' feces, and it is considered to be the classic method that includes detection of the number of bifidobacteria, lactobacilli, Enterobacteriaceae, Escherichia Coli, Proteus, enterococci, Staphylococcus Aureus, Pseudomonas Aeruginosa, Candida [16 - 19]. The severity of dysbacteriosis is determined by the degree of reduction in bifidobacteria and other obligate microflora and increasing in the number of opportunistic species.

Objective and tasks: the aim of this study was to analyzes the overall structure of microbiota in patients with adenocarcinoma of colorectal and the stomach and healthy controls.

Materials and methods of the study. In the study of this issue, an analysis of the immediate results of treatment of 74 patients, which was a test group, which operative treatment was performed in a radical volume. Distribution of patients depending on the stage – T2-3 N0 M0 – 17.5%, T2-3 N1-2M0 – 73.2%, T4 N1-2M0 – 9.3%. Most patients (77%) had adenocarcinoma. Squamous cell carcinoma has been found in 13.5% of cases. In all patients, the diagnosis of cancer was morphologically verified before surgery. To clarify the extent of intestinal and bowel dysbiosis conventional bacteriological method was used to determine obligate, opportunistic, and pathogenic microflora. Metabolites of good microbiocaenosis such as acetic, propionic, butyric, lactic, oxalic, and bad α-ketoglutaric, phenyl propionic acids, n-cresol, skatol, indol were studied by gas-liquid chromatography on a chromatograph Tsvett 1000. Chromatography was performed on glass column 300x 0.3 al. The column and the evaporator temperature was 40 °C. The gas (argon) rate was 25ml/min. Identification of metabolites in feces extracts was carried out by the method of R.N Makeeva et al. Amines (methylamine, histamine, serotonin) were studied with the help of high-performance liquid chromatography.

Statistical processing of the obtained data was performed using nonparametric methods (Spearman's rank correlation coefficient, rS), Wilcoxon's Criteria (homogeneity of the studied features).

Results and Discussion. The studies suggested that colorectal cancer and stomach cancer develops from the complex interactions between inherited susceptibility and environmental factors, there a strong association between adenomatous polyps and some pathogenic bacteria are the precursors of the vast majority of colorectal cancers and stomach (figure 1).

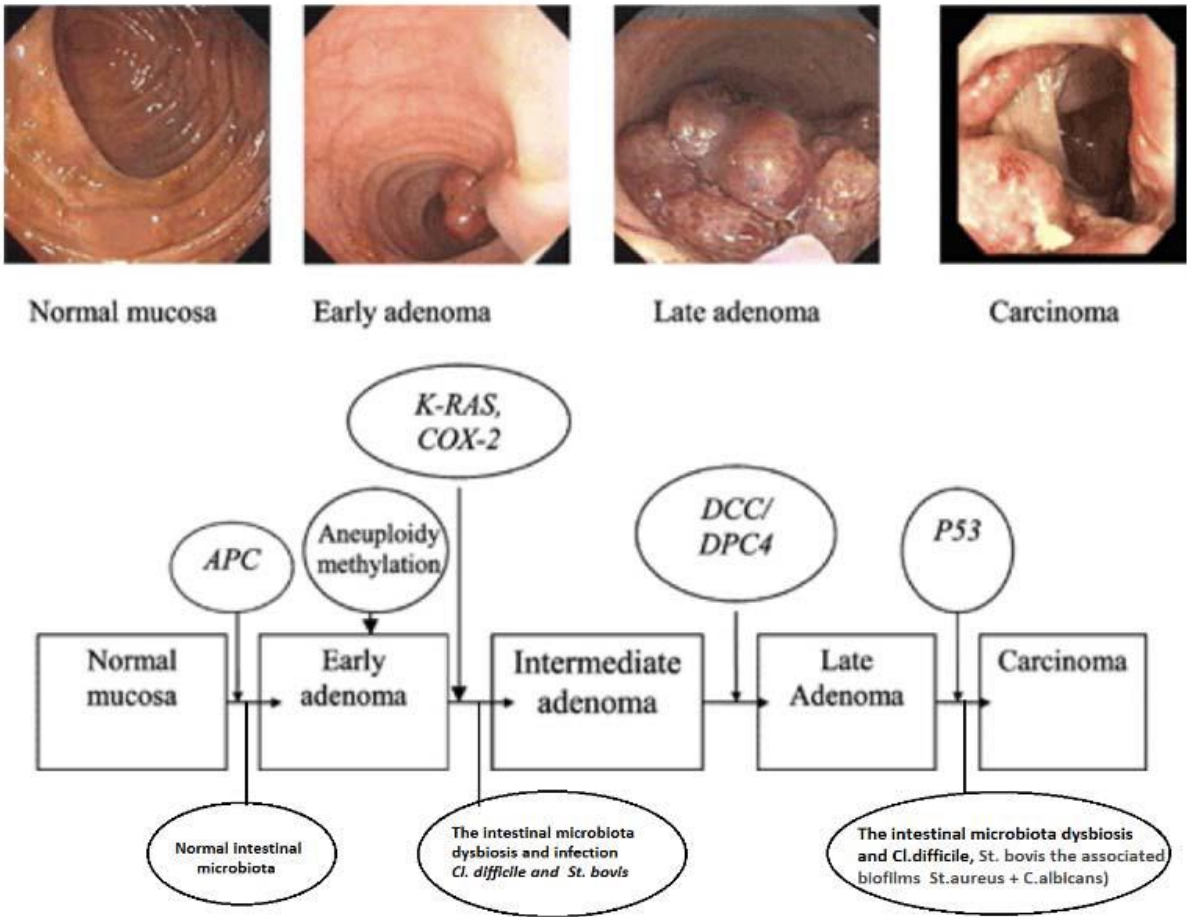

Figure 1. The complex interactions between inherited susceptibility and environmental factors

The gastrointestinal tract is known to be a complex and finely balanced ecosystem. It is one of the largest interfaces between the outside world and the human internal environment. The gut microbiota is highly vulnerable to changes in the gut microenvironment. Identification of the patients with gastric cancer and high titers of Clostridium Difficile should be considered as a mechanism leading to Clostridium Difficile expansion and subsequent infection.

We have suggested a potential relationship between increased fecal carrier levels of Streptococcus Bovis and human gastrointestinal disease, IBD, and primarily colonic cancer and stomach cancer in adult patients (Table 1).

Table 1.

The potential relationship between increased fecal carrier levels of *Streptococcus Bovis* and human gastrointestinal disease, IBD, and primarily colon cancer and stomach cancer in adult patients

Probably Causative	*Probably Protective*
• A high-fat and low-fiber diet • Red meat consumption • Low physical activity • Obesity	• Aspirin, NSAIDs, and cyclooxygenase-2 inhibitors • Calcium • Hormone replacement therapy (estrogen) • Low body mass • Physical activity • Life style • Diet
Probably Causative	*Probably Protective*
• Beer and ale consumption • Cigarette smoking • Diabetes mellitus • Environmental carcinogens and mutagens • Low dietary selenium • The intestinal microbiota dysbiosis and *Clostridium Difficile, Streptococcus Bovis and associated biofilms the Staphylococcus Aureus + Clostridium Albicans)*	• Carotene-rich foods • High-fiber diet fruits and vegetables • Vitamins C, E, and D • Yellow-green cruciferous vegetables • Diet • Low sugar and carbohydrate consumption • Possible antimicrobial

Investigation of metabolic parameters of microflora in patients with adenocarcinoma found a significant decrease of the content of carbon acids, such as acetic (at 73,5%), propionic (at 61 %), butyric (at 65,5 %), and lactic (at 76,2%). It is consistent with a decrease in the number of protective anaerobic microflora (Bifidobacteria, Bacteroides, Lactobacilli). There was detected a decrease in physiological levels α-ketoglutaric and oxaloacetic acids at 50.5% and 60,8% respectively, which confirms the weak in the biochemical activity of both aerobic and anaerobic intestinal bacteria, especially in carbohydrates metabolism (Table 2).

Evaluation of aromatic amino acids derivatives ratio in fecal extracts showed increased activity of bowel microflora. The profile of fecal compounds was characterized by an increase in the total number of aromatic substrates (at 325 %), in a quantity of n-cresol (at 347 %), indole (at 405 %), and phenyl propionic acid (at 264 %). Determination of methylamine, histamine, and serotonin showed increasing of decarboxylase activity of that microflora according to cyclic amino acids (histidine, tryptophan). Comparison of amines

profile in patients with adenocarcinoma and group of conventionally healthy people found an increase in the concentration of methylamine (at 621%), histamine (at 710 %), and serotonin (at 373 %).

Table 2

Metabolic activity indexes of colon microflora in patients with adenocarcinoma

Metabolic indexes, (mg/ L)	Group, M±m	
	Patients with adenocarcinoma	Conventionally healthy people
Carbon acids: - acetic	345,62±15,38*	1385,37±18,82
- propionic	78,43±6,25*	194,72±16,43
- buturic	54,27±4,52*	143,86±9,26
- lactic	86,33±7,14*	382,40±12,34
Dicarbon acids: - α-ketoglutaric	68,34±4,92*	140,15±8,62
- oxaloacetic	8,33±6,15	19,37±2,14
Aromatic substrates: - n-cresol	5,44±0,26*	1,14±0,04
- indol	6,18±0,35*	1,32±0,03
- scatol	6,53±0,42*	1,27±0,02
- phenylpropionic acid	4,96±0,37*	1,15±0,012
Amines: - methylamine	2,53±0,22*	0,32±0,014
- histamine	2,66±0,18*	0,28±0,018
- serotonine	8,24±0,57*	1,62±0,15

Note: * differences are significant p <0,05

It should be noted that in the conventionally healthy group an increased quantity of methylamine, histidine, serotonin up to levels of patients with adenocarcinoma was established in 3 patients (7%) and indexes of n-cresol, indole, skatole were increased in 4 patients (9.3 %). Such results showed the increased biochemical activity of microflora and the development of putrefactive processes and suggested to include such patients at risk of a possible development of adenocarcinoma pathology.

Thus, the data suggested the development of intestinal dysbiosis in patients with adenocarcinoma, which was characterized by inhibiting obligate protective microflora and activation opportunistic microorganisms on the base of carbohydrates, fats, and proteins metabolism disturbances and accumulation of toxic metabolic products that may be an important pathogenetic factor of tumor tissue activation, induction, proliferation, and metaplasia. Significant accumulation of biogenic amines (methylamine, serotonin, histamine) is a leading metabolic profile of microflora inpatient with adenocarcinoma and may have a

predictive value for diagnosis, pathogenetic therapy, and determination of food nutrients role in the mechanisms of cancer formation, as well as identifying populations of cancer risk.

Conclusion

We first found that a significant difference in intestinal and bowel bacterial flora existed between healthy individuals and colorectal carcinoma and stomach cancer patients. We demonstrated that the composition of the tumor microbiome differed from that of adjacent non-neoplastic tissue. We also determined the subsite-specific alterations in the colorectal carcinoma and stomach cancer microbiota. There is a high incidence of colorectal cancer and stomach cancer associated with Streptococcus Bovis. The results of these studies provide evidence supporting that these bacteria could be used for microbiota-associated diagnosis, prognosis prevention, and treatment for colorectal carcinoma and stomach cancer. These results suggested that the mucosa-associated microbiota is dynamically associated with colorectal carcinoma, which may provide evidence for microbiota-associated diagnostic, prognostic, preventive, and therapeutic strategies for colorectal carcinoma and stomach cancer.

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