REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Multi-omics analysis of human gut microbiota in colorectal cancer patients

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Received on: 07. 08. 2022

Revised on: 27. 08. 2022

Accepted on: 04. 09. 2022

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Abstract

Colorectal cancer (CRC) is a critical health problem. It is the second cause of death globally and the third world's most frequently diagnosed. Multiple evidences and suggestion imply the relationship of gut microbiota and colorectal cancer carcinogenesis. The different omics techniques like, metagenomic, metaproteomic and metabolomic approaches have led to important advances in the study of the intestinal microbiome, the host as well as the intestinal environment. Different bacterial species, proteins and metabolites have a crucial role in colorectal cancer screening, detection and recurrence. Integration of these three omics analysis in drawing attention to reveal taxonomic and functional structure of human gut microbiota in addition several protein and metabolites detection had helped in construction of microbial communities and their diversity in colorectal cancer patients and healthy controls besides that these bacterial species, metabolites and proteins could be used as a critical biomarkers candidate in colorectal cancer detection, prognosis and recurrence.

Keywords: Colorectal cancer, Metagenomic, Metaproteomic, Metabolomic, Gut microbiota.

1. Introduction:

Colorectal cancer is the third most common cause of cancer mortality in the world (Jemal et al., 2011). The disease typically develops over many years via a sequence of genetic changes, known as the adenoma–carcinoma sequence (Fearon & Vogelstein, 1990). Chronic inflammation has been proposed to be involved in the promotion of cancer (Grivennikov, 2012). Gut microbiota dysbiosis as well as increased intestinal permeability are highly linked to colon inflammation, which could be the key factor for initiation and/or progression of CRC (Dzutsev et al., 2014).The association of the microbiota with cancer has recently been discussed, highlighting that different cancer types present distinct microbial signatures. Surprisingly, bacteria is found to be present inside the tumor cells in addition to immune cells of cancer patients (Nejman et al., 2020) .In Egypt, several studies showed that there is a relationship between the bacterial communities presents in the body and the impact of these communities on the development of the disease condition, whether positive or negative effect (Salah et al., 2019; Ali, M. S, 2019;Ramadan et al., 2019A;Ramadan et al., 2019B;Ramadan et al., 2021; Elsherbiny et al., 2022).

Colonoscopy, the standard screening method for CRC diagnosis. However, it is risky and relatively costly (Eklöf et al., 2017) Besides that colonoscopy presents a level of patients discomfort , being

invasive, and poses some health risks like post polypectomy, colon- puncture, in-traperitoneal bleeding and possibility of infection(Toma et al., 2018; Zhang et al., 2019; Löwenmark et al., 2020). Thus, the need for other detection techniquesthat have both benefit of being non-invasive and high effectiveness. Fecal tests remain the focus of attention for non-invasive strategyin CRC diagnosis (Toma et al., 2018).

Role of bacterial species in CRC

Beside the shift in microbiota composition, pathogenic bacterial species may also have a role in the development of CRC. There are different pathogenic microbes associated to the promotion of CRC, such as several Bacteroides species (*B. vulgatus*, *B.fragilis* and *B. stercoris*), Bifidobacterium species (*B. angulatum*), Ruminococus species, Fusobacteriumprausnitzietc (Moore & Moore, 1995). All these microbes may cause CRC tumorigenesis by inducing proliferation of the epithelial cells,thus producing damage in the epithelial barrier, and causing inflammation. Moreover, different toxins may damage DNA inducing pro-tumorigenic effect. For example, *Bacteroides fragilis* toxin is known to activate Wnt and NF-kB signaling pathways and enhance epithelial release of pro-inflammatory molecules (Wu et al., 2006; Goodwin et al., 2011), *Fusobacteriumnucleatum* has emerged as a crucial candidate for CRC predisposition, due to its ability to bind to E-cadherin on the surface of colon cells by FadA adhesion, causing activation of Wnt/B-catenin signaling pathway and production of an inflammatory and oncogenic response (Rubinstein et al., 2019) and able to bind to the inhibitory immune receptor by Fap2 adhesin results in alteration of natural killer cells (Brennan & Garrett, 2018).

Next-generation sequencing

Next-generation sequencing (NGS) allowed the use of genomic approaches to better understand the complex microbial environment from various biological samples, providing a comprehensive overview of the taxonomic and functional potential of microbial community (Mandal et al., 2015). Metagenomic studies have most commonly used one of two main approaches to assess the composition of microbiomes: whole-genome shotgun (WGS) sequencing, and 16S ribosomal RNA amplicon sequencing.16S sequencing is the lack of taxonomic resolution; while the variable regions of the gene are particular to different organisms, finding differences within this section of a few hundred base pairs versus differences across the entire genome can often limit identification to only the genus level. In contrast, WGS allows for more accurate detection of species/ strains, and diversity within samples, as well as identification of the coding potential of the genome, which can only be indirectly inferred in the case of 16S sequencing, by extrapolation from known genomes (Ranjan et al., 2016) and provide information on the genes encoded by the strains present in the sample, thus this information can be used to reconstruct potential metabolic capcities of microbial ecosystem (Saus et al., 2019).By using knowledge of WGS many bacterial taxa have been shown to have differential abundance among CRC patients in comparison to healthy controls (Zuo et al., 2022).

Nowadays next generation sequencing technology by Illumina platform offers several sequencing machines with low error rate and cost of sequencing. Although Illumina produces shorter reads, it is able to read the DNA from either end and to connect these forward and reverse called pair-end (PE). This platform is used in both targeted sequencing metagenomic for the length of connected reads and whole metagenome shotgun sequencing (WMS) approach for its higher throughputand low cost (Qin et al., 2010). The major bottleneck in metagenomic sequencings is this method don't distinguish among active, dead, and dormant cells (Burkert et al., 2019).

Proteomics

Proteomics, initially defined as the study of all proteins expressed by a single organism. In respect to analysis of the protein content of the microbial communities, such as gut microbiota is called ''metaproteomics" (Schneider & Riedel, 2010). Proteomics is a powerful technique aimed to find differences in protein expression between healthy and disease states (Álvarez-Chaver et al., 2018),

A metaproteomic analysis include four steps: firstly, extraction and purification of proteins, secondly, enzymatic digestion of proteins into peptides, third, separation of peptides, usually by chromatography, followed by mass spectrometric analysis and finally protein identification by database sequence comparison (Petriz et al., 2017; Lee et al., 2017).Metaproteomics workflow typically compromise sample collection, protein extraction, fractionation, mass spectrometry (MS) analysis and database searches. In human gut microbiota study, fecal samples are commonly employed to characterize global proteome of the entire gut (Kolmeder and Vos, 2014). To date, MS remains as the analytical platform of choice for metaproteomics. Beyond protein identification,

quantitative analysis is important to determine key microbial players that contribute to metabolic functions (von Bergen et al., 2013)

Metaproteomics is a rising technique but has some disadvantages related to the complexity of the sample, including both the complexity of the matrix as well as the microbial community itself, generation of numerous false positives from the use of large database and data interpretation is considered as a major drawback for metaproteomic analysis (Zhang et al., 2018).

Overall, metaproteomic study is gradually gaining the power to reveal the functionality of the complex microbial consortium, from understanding the role of microbiota in healthy individuals as well as diseased (Lee et al., 2017).The major benefit of Proteomic studies is generate a large protein databases hence, expanding list of protein biomarkers as a potential candidate that are differentially expressed in CRC patients (Álvarez-Chaver et al., 2014).

Metabolomics

Metabolomics is concerned with the high-throughput identification, quantification and characterization of the small molecule metabolites in the metabolome (Johnson et al., 2016).Metabolomics study is typically classified into two categories: targeted and untargeted metabolomics. In targeted metabolomics, clearly defined and selected compounds are analyzed and compared from different sample groups. This approach involves the measurement of identified and chemically defined compounds, which is related to the metabolic pathways. On the other hand, untargeted metabolomics mainly focuses on the global consideration of both known and unknown metabolites for comprehensive analysis to detect and figure out an alteration from different conditions in order to identify and relatively quantify the metabolites with different contributions in terms of classification. However, identification of significant peaks remains a challenge, complicating in-depth mechanistic or biochemical understanding (Bingol, 2018). Liquid chromatography coupling to mass spectrometry (LC-MS) is more widely used for the analysis of both non-polar metabolites (Chetwynd et al., 2019) and polar metabolites (Röth et al., 2019).

The human colon harbors the densest metabolically active microbial community in the body. Over the past decades, several categories of gut microbial specific metabolites have been identified, including short chain fatty acids (SCFAs),secondary bile acids, polyamines, indoles, vitamins etc(Yan et al., 2016). Several evidences indicate microbiota-derived metabolites exert an important effect on host physiology and diseases prognosis (Louis et al., 2014).

The concentration of SCFAs varies along the intestinal tract, with the highest levels in the cecum and proximal colon, and its levels decrease in the distal colon due to absorption by colonic epithelial cells. Butyrate is one of the chief energy sources for local colonic epithelial cells, while the majority of acetate and propionate enter the circulation to exert systemic effects that influence various pathological conditions (Den Besten et al., 2013).Only a small amount of unabsorbed SCFAs are detected in fecal samples, Due to extensive absorption (Van der Beek et al., 2017).

Bile acids, which are primarily produced in the liver, are metabolized to secondary bile acids by the gut microbiota in the intestinal tract, these Secondary bile acids, especially deoxycholic acid (DCA), are considered a critical contributor to the development of CRC (Ticho et al., 2019).

The intestinal tract contains high levels of polyamines (PAs), mainly including putrescine, spermidine and spermine that are obtained from diet or biosynthesized by bacteria and host (Rooks and Garrett, 2016). PAs are essential to cell proliferation as well as immune cell differentiation and activation (Pegg, 2016). Bacterial pathogens, such as *Escherichia coli*, *Helicobacter pylori*, and *Shigellaflexneri*, rely on polyamines for their virulence (Shah &Swiatlo, 2008).

Metabolomics aimed to identify biomarkers and the affected metabolic pathwaysby revealing the differences in the identified differential metabolites in CRC versus healthy controls (Amir et al., 2021).

Conclusion

In conclusion, many microbial species as well as microbial-derived proteins and metabolites profoundly affect colon tumorigenesis and increase cancer risk. Metagenomic, metaproteomic and metabolomic data can be integrated to provide insight into the functioning of bacterial communities in the gut of CRC patients and to identify a potential biomarker candidates.

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