

Analysis of the potential correlation between gastric cancer and gastrointestinal microbiota via in-silico data mining

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Research

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Abstract

Background

Emerging evidence shows the gastrointestinal microbiome might play an important role in the carcinogenesis of gastric cancer. While *Helicobacter pylori* has been reported to be a specific risk factor of gastric cancer, it is still controversial whether significant difference of non- *H. pylori* microbiota exists between gastric cancer patients and healthy control.

Results

In this study, we employed multiple bioinformatic databases to excavate the potential correlation between gastrointestinal microbiome and gastric cancer. The databases involved in this investigation include HMDB, STITCH, OMIM, GWAS Catalog, WebGestalt, Toppgene, GeneMANIA. In addition, the network diagrams were built by use of Cytoscape software. Notably, our results showed that 33 common genes participate in both gastrointestinal microbiome and gastric cancer. The further analysis of these common genes suggested that there was a wide array of interactions and pathways in which the correlation between gastrointestinal microbiome and gastric cancer is involved.

Conclusions

Our present study gives a bioinformatic insight into possible pathways in which the gastrointestinal microbiome play roles in gastric cancer. Future efforts are necessary to be paid to elicit the exact mechanisms as well as potential therapeutic targets of gastric cancer.

Introduction

The gastric cancer (GC) accounts for 723,000 deaths annually in both sexes worldwide, being the third leading cause of cancer-related death. With devastating prognosis, the gastric cancer is becoming a heavy burden as well as a threat to human health (1).

The majority of microbes in our bodies reside in the gut with a profound influence on human physiology and pathology. The gene set of gut microbiota is 150 times larger than the human gene complement (2). The gastrointestinal (GI) microbiota is crucial for the maintenance of human health, involving in energy metabolism (3), nutrient absorption (4) and the immune function (5). The change of the microbiota balance, which is called dysbiosis, contributes to the aberrant variation of microbiota diversity and abundance followed by the development of various disease, including gastrointestinal disease such as gastroesophageal reflux disease, gastritis and pseudomembranous colitis (6), metabolism disease such as obesity (7) and diabetes (8), cardiovascular disease(9), disorder of immunity(10), neuropsychiatric disease such as Parkinson disease, anxiety and autism spectrum disorder (11,12), cancer-related disease such as hepatic carcinoma (13), colorectal carcinoma (14), pancreatic carcinoma (15,16), breast cancer (17) and melanoma (18,19).

The infection of *Helicobacter pylori* (*H.pylori*) is the strongest known risk factor for the development of gastric cancer (20,21). Though very low proportion of the cases infected *H. pylori* progressed to gastric cancer (22), the eradication of *H. pylori* indeed contributes to a decrease of the incidence of gastric cancer (23). However, it is under controversial that whether the non-*H.Pylori* microbiota have a significant role in gastric cancer development.

Dicksved et al. investigated patients with gastric cancer and dyspeptic controls. By use of the molecular profiling approach terminal restriction fragment length polymorphism and 16S rRNA gene cloning and sequencing, they found no significant difference between the gastric bacterial community from two groups. Sequencing of 140 clones revealed 102 phylotypes, with representatives from five bacterial phyla (Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria), while the abundance of *H.pylori* is relatively low (24). In non-*H.pylori* gastric cancer cases, *Helicobacter cinaedi*, *Helicobacter mustelae* and *Campylobacter hyointestinalis* were detected (25), while no significant role of these non-*H.pylori* bacteria in gastric cancer (26).

Some research suggest that a gradual shift of gastric microbiota community is likely to take place in the progression from precancerous lesions to gastrointestinal cancer, while different studies do not reach a consensus on the nature of the shift. Both increases (27,28) and decreases (29) in the bacterial diversity have been reported through comparing samples in each step of the gastric carcinogenesis, from chronic gastritis or intestinal metaplasia to gastric cancer. The increase in *Lachnospiraceae* and members of the *Lactobaccillaceae* and *Streptococcaceae* family during the carcinogenesis were turned out to be the most consistent finding among the studies. Nevertheless, the exact role of these non-*H.pylori* bacteria in gastric carcinogenesis as well as the factors regulating the microbiota diversity are still remains to be determined. The discrepancy between the methods of different studies make it difficult to directly compare the results.

In this study, we employed multiple bioinformatics databases to further explore the possible association between gastrointestinal microbiota and gastric cancer in the level of interaction genes. Our data will help to provide several new insights for further investigation of the role of the gastrointestinal microbiota in the development of gastric cancer.

Results

1.

Analysis of the common genes shared by GI microbiota and GC

1.1

The identification of the genes related to GI microbiota

10 metabolites of GI microbiota associated with gastric diseases were identified according to the HMDB database. These metabolites included Trimethylamine N-oxide, Butyric acid, Indoleacetic acid, Propionic acid, Indoxyl sulfate, p-Cresol, Acetone, Methane, 2-Methylerythritol and Glycine. 313 human genes

associated with the 10 metabolites were identified by searching the STITCH database. The number of genes related to each metabolite is shown in Table 1.

Table 1
The metabolites of GI microbiota and associated gene number

HMDB ID	Name	Genes
HMDB0000925	Trimethylamine N-oxide	36
HMDB0000039	Butyric acid	54
HMDB0000197	Indoleacetic acid	57
HMDB0000237	Propionic acid	59
HMDB0000682	Indoxyl sulfate	27
HMDB0001858	p-Cresol	25
HMDB0001659	Acetone	51
HMDB0002714	Methane	23
HMDB0011659	2-Methylerythritol	9
HMDB0000123	Glycine	49

1.2

The identification of the genes related to GC

464 genes from OMIM database and 34 genes from GWAS Catalog were shown to associated with GC. No overlap was found in genes obtained from the two databases.

1.3

The common genes shared by GI microbiota and GC

33 genes were identified to be the common genes shared by GI microbiota and GC. The symbol and description of each gene is shown in Table 2.

Table 2
The common genes shared by gastric cancer and gastrointestinal
microbiome

Gene	Full name of gene
TRH	thyrotropin releasing hormone
GNA11	G protein subunit alpha 11
GNA12	G protein subunit alpha 12
GNA14	G protein subunit alpha 14
MTRNR2L2	MT-RNR2 like 2
GNAI1	G protein subunit alpha i1
GNAI3	G protein subunit alpha i3
GNAO1	G protein subunit alpha o1
MTOR	mechanistic target of rapamycin kinase
GNAT1	G protein subunit alpha transducin 1
GNAT2	G protein subunit alpha transducin 2
GNAT3	G protein subunit alpha transducin 3
BCAS2	pre-mRNA-splicing factor
GNB1	G protein subunit beta 1
GNB2	G protein subunit beta 2
GNB2L1	guanine nucleotide-binding protein subunit beta-2-like 1
GNB3	G protein subunit beta 3
GNB4	G protein subunit beta 4
TRIO	trio Rho guanine nucleotide exchange factor
GNG2	G protein subunit gamma 2
GNG3	G protein subunit gamma 3
GNG4	G protein subunit gamma 4
GNG5	G protein subunit gamma 5
GNG7	G protein subunit gamma 7
TRIT1	tRNAisopentenyltransferase 1

Gene	Full name of gene
GNGT1	G protein subunit gamma transducin 1
GNGT2	G protein subunit gamma transducin 2
RSP01	R-spondin 1
GNG10	G protein subunit gamma 10
GNG11	G protein subunit gamma 11
GNG12	G protein subunit gamma 12
GNG13	G protein subunit gamma 13
PCSK9	proprotein convertase subtilisin/kexin type 9

2.

Gene ontology analysis of the common genes shared by GI microbiota and GC

Gene ontology analysis of 33 common genes shared by GI microbiota and GC conducted by WebGestalt is shown in Fig. 2. The main biological process in which the 33 common genes enriched include cell communication, response to stimulus, biological regulation, multicellular organismal process, metabolic process, cellular component organization and location. The main molecular function of these genes include protein binding, hydrolase activity, nucleotide binding, ion binding, nucleic acid binding, transferase activity and molecular transducer activity. The main cellular components that these genes enriched in include membrane, macromolecular complex, vesicle, cell projection, vacuole, cytosol, nucleus, endomembrane system and cytoskeleton.

3.

Pathway analysis of the common genes shared by GI microbiota and GC

The pathway analysis was conducted by WebGestalt and ToppGene. The pathway enrichment analysis results are shown in Supplementary Table 1. The pathways of the common genes shared by GI microbiota and GC involve G-protein activation, Opioid Signalling, Protein folding, Platelet activation, signaling and aggregation, ADP signalling through P2Y purinoceptor 12, Regulation of insulin secretion, Morphine addiction, Retrograde endocannabinoid signaling, Glutamatergic synapse, Dopaminergic synapse, Apelin signaling pathway, Thromboxane signalling through TP receptor, ADP signalling through P2Y purinoceptor 1, Muscarinic acetylcholine receptor 2 and 4 signaling pathway, PI3K-Akt signaling pathway, Signal amplification, Cooperation of PDCL (PhLP1) and TRiC/CCT in G-protein beta folding, Thrombin signalling through proteinase activated receptors (PARs), Chaperonin-mediated protein folding, Prostacyclin signalling through prostacyclin receptor, G alpha (z) signalling events, G beta:gamma signalling through PLC beta, Adrenaline, noradrenaline inhibits insulin secretion, Presynaptic function of Kainate receptors, Glucagon-type ligand receptors, Opioid proopiomelanocortin pathway, Activation of

Kainate Receptors upon glutamate binding, Opioid prodynorphin pathway, Ca²⁺ + pathway, Enkephalin release, Cholinergic synapse, GABAergic synapse, Circadian entrainment, GABA B receptor activation, Activation of GABAB receptors, Glucagon-like Peptide-1 (GLP1) regulates insulin secretion, Corticotropin releasing factor receptor signaling pathway, 5HT1 type receptor mediated signaling pathway, Vasopressin regulates renal water homeostasis via Aquaporins, Metabotropic glutamate receptor group II pathway, Opioid proenkephalin pathway, Integration of energy metabolism, Serotonergic synapse, G beta:gamma signalling through PI3Kgamma, Endogenous cannabinoid signaling, G alpha (12/13) signalling events, Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction, G-protein beta:gamma signaling, Aquaporin-mediated transport, GABA receptor activation, Thyrotropin-releasing hormone receptor signaling pathway, Histamine H1 receptor mediated signaling pathway, G alpha (s) signalling events, Neurotransmitter Receptor Binding And Downstream Transmission In The Postsynaptic Cell, 5HT2 type receptor mediated signaling pathway, G alpha (q) signalling events, G alpha (i) signalling events, Glucagon signaling in metabolic regulation, Histamine H2 receptor mediated signaling pathway, GABA-B receptor II signaling, G protein gated Potassium channels, Inhibition of voltage gated Ca²⁺ + channels via Gbeta/gamma subunits, Activation of G protein gated Potassium channels, Oxytocin receptor mediated signaling pathway, Beta3 adrenergic receptor signaling pathway, Alcoholism, Beta-catenin independent WNT signaling, 5HT4 type receptor mediated signaling pathway, Inwardly rectifying K⁺ channels, Platelet homeostasis, Class B/2 (Secretin family receptors), Transmission across Chemical Synapses, Chemokine signaling pathway, Muscarinic acetylcholine receptor 1 and 3 signaling pathway, Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway, Pathways in cancer, Beta1 adrenergic receptor signaling pathway, Beta2 adrenergic receptor signaling pathway, GPCR downstream signaling, Signaling by GPCR, Signaling by Wnt, Neuronal System, Hemostasis, Gastrin-CREB signalling pathway via PKC and MAPK, PI3 kinase pathway, Angiotensin II-stimulated signaling through G proteins and beta-arrestin, Ras signaling pathway, Metabotropic glutamate receptor group III pathway, Potassium Channels, Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway, GPCR ligand binding, Dopamine receptor mediated signaling pathway, Wnt signaling pathway, Metabolism of proteins, Sphingosine 1-phosphate (S1P) pathway, Transmembrane transport of small molecules, S1P2 pathway, S1P3 pathway, Inflammation mediated by chemokine and cytokine signaling pathway, PLC beta mediated events, G-protein mediated events, S1P5 pathway, Alzheimer disease, S1P4 pathway, Long-term depression, Phototransduction, CXCR3-mediated signaling events, Thrombin signaling and protease-activated receptors, Activation of the phototransduction cascade, Bioactive Peptide Induced Signaling Pathway, Chagas disease (American trypanosomiasis), Inhibition of adenylate cyclase pathway, Adenylate cyclase inhibitory pathway, S1P1 pathway, Synthesis, secretion, and inactivation of Glucagon-like Peptide-1 (GLP-1), Aspirin Blocks Signaling Pathway Involved in Platelet Activation, Incretin synthesis, secretion, and inactivation, Visual signal transduction: Cones, Visual signal transduction: Rods, CXCR4 Signaling Pathway, Intracellular Signalling Through Prostacyclin Receptor and Prostacyclin, Vasopressin Regulation of Water Homeostasis, Intracellular Signalling Through PGD2 receptor and Prostaglandin D2, Phospholipids as signalling intermediaries, Nicotine pharmacodynamics pathway, Excitatory Neural Signalling Through 5-HT₄ and Serotonin, Corticotropin Activation of Cortisol Production, PKC-catalyzed phosphorylation of inhibitory phosphoprotein of myosin phosphatase, How Progesterone Initiates Oocyte

Membrane, Signaling Pathway from G-Protein Families, Inactivation, recovery and regulation of the phototransduction cascade, Excitatory Neural Signalling Through 5-HTR 7 and Serotonin, The phototransduction cascade, and Rapid glucocorticoid signaling.

4.

Analysis of interaction networks of the common genes shared by GI microbiota and GC

The 33 common genes shared by GI microbiota and GC were further analyzed by GeneMANIA, and the interaction networks were mapped by Cytoscape software (Fig. 3). Each node represents a particular gene. The color of the gene represents a kind of biological function. Here show the 7 main functions of the common genes, including heterotrimeric G-protein complex, extrinsic component of cytoplasmic side of plasma membrane, G-protein beta/gamma-subunit complex binding, extrinsic component of plasma membrane, extrinsic component of membrane, GTPase activity, response to glucagon. Each line of a color indicates a kind of interaction. The detail of the interactions is in Table 3.

Table 3
The interaction patterns and their proportions of protein

Network Categories	Percentage
Shared protein domains	45.34%
Physical Interactions	34.19%
Predicted	12.40%
Pathway	6.50%
Co-expression	1.21%
Co-localization	0.36%

Databases And Methods

Multiple bioinformatic approaches were used to analyze the correlation between gastrointestinal microbiota and gastric cancer. All analyses were updated on May 31st, 2018. The databases and software adopted in this study contain HMDB, STITCH, OMIM, GWAS Catalog, WebGestalt, Toppgene, GeneMANIA and Cytoscape.

Since the enormous quantity of the species in human gastrointestinal tract and species vary across individuals, it is of great difficult to search genes related to gastrointestinal microbiota directly. We employed an indirect approach to take the metabolites of gastrointestinal microbiota as the intermediary. The genes associated with gastrointestinal microbiota metabolites were utilized as that related to gastrointestinal microbiota. The bioinformatic analytic procedures are shown in Fig. 1.

HMDB, which is short for Human Metabolome Database (<https://www.hmdb.ca>), is a comprehensive internet resource focusing on metabolism and human metabolites and facilitating research on human metabolic science. Information in this database include the biological functions, physiological abundance, clinical connection, biochemical reaction, pathways of metabolism and reference spectra of complicated human metabolites(30).

STITCH, which is short for Search Tool for Interacting Chemicals (<https://stitch.embl.de>), mainly functions to integrate the interactions between small molecules and proteins. The database facilitates the users to obtain a view of the potential interaction network and effects of chemicals. The users are allowed to get the information on the binding affinities between proteins and chemicals and filter the tissue-specific proteins (31).

OMIM, which is short for Online Mendelian Inheritance in Man (<http://omim.org>), is a human Mendelian genetic-associated database mainly focusing on the human genes and phenotypes. The information of OMIM is based on published peer-reviewed biomedical literature and applied widely around in biomedical field(32).

GWAS Catalog, which is short for Genome-wide association studies Catalog (www.ebi.ac.uk/gwas), is an open-access collection of all the published genome-wide association studies (GWAS) and related results(33).

WebGestalt, which is short for WEB-based Gene SeT AnaLysis Toolkit (<http://www.webgestalt.org>), is a web tool mainly applied to conduct functional enrichment analysis, revealing the biological information of the high- throughput omics data (34).

ToppGene Suite (<http://toppgene.cchmc.org>) is a free and open access database providing information on gene list network enrichment, candidate gene prioritization and the identification and prioritization of candidate genes of novel disease (35).

GeneMANIA (<http://genemania.org>) is a widely used web application to predict gene function, analyze gene lists and prioritize genes for functional assays. Based on the given query gene list, GeneMANIA search for the functionally similar genes, while a single query gene could generate genes with similar functions (36).

Cytoscape (www.cytoscape.org/) is designed to facilitate the visualization of molecular network and biological pathways. The software is able to depict large networks with more than ten thousand nodes and edges and display interactions between distinct molecular network constituents. The generation of biological networks are supported by the application of the editor module (37).

Discussion

The overlap of the genes related to GI microbiota and gastric cancer indicates that a probable correlation between GI microbiota and gastric cancer might exist. The further GO and pathway analysis suggest that

the correlation is likely to be involved in widespread aspects which contain sophisticated interactions and mechanisms. The analysis of the 33 common genes will provide insight into further investigation into the mechanism of the gastric carcinogenesis and the potential diagnostic and therapeutic target of gastric cancer.

The main functions of the 33 common genes shared by GI microbiota and GC include GTPase activity, nucleoside-triphosphatase activity, pyrophosphatase activity, hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides, hydrolase activity, acting on acid anhydrides, G-protein beta/gamma-subunit complex binding, signal transducer activity, G-protein coupled receptor binding, G-protein coupled serotonin receptor binding, GTP binding, guanyl ribonucleotide binding, guanyl nucleotide binding, signaling receptor binding, protein-containing complex binding, GTPase activating protein binding, purine ribonucleoside triphosphate binding, purine ribonucleoside binding, ribonucleoside binding, nucleoside binding, purine ribonucleotide binding, purine nucleotide binding and ribonucleotide binding.

The main pathways that 33 common genes enriched in mainly focus on G protein related pathways, several cell signal transduction pathways and synapse involving pathways. What is more, several rare pathways attracted our attention, such as opioid signaling and morphine addiction, the regulation of insulin secretion and inhibition as well as protein folding.

Opioids such as morphine have been taken advantage in analgesia for centuries. Data from 1980s drew attention on the effect on the growth of tumors. However, studies either in vivo or in vitro in order to elicit the effects of opioids shows conflicting results, making it still remains to be ascertained that whether the effects are beneficial or harmful. Though the analgesic effect of opioids is indispensable in clinical administration of tumor pain, opioids is also considered to be menacing for patients with tumor on account of the extra analgesia effects such as immune suppressive effects and angiogenic potential(38). Efforts have been paid to explore the correlation of gastric cancer and the administration of morphine. Xie et al. developed experiments to effects of the treatment of exogenous morphine on gastric cancer cell line MGC-803. The results showed that the treatment of morphine led to the significant inhibition of cell growth and proliferation as well as cell cycle arrest in the G2/M phase(39). Some other studies connected the morphine administration with gastric microbiota. It was reported that opioid induce gastric microbial disruption and bile dysregulation which further compromise the gastric barrier (40). Opioid is also reported to involve in the exacerbation of gram-positive sepsis which could be rescued by IL-17A neutralization (41). While the morphine and other narcotics have been noted to alter the composition of GI microbiota and promote the translocation of GI microbiota, it is reported that GI microbiota was indicated to play an essential role in modulating the response to chronic morphine administration(42). The opioids along with their effects on GI microbiota is also reported to have a role in the neuroinflammation in the central nervous system that is mediated by a gut-brain signaling axis, which further contributed to several inflammation-related psychopathologies. The difference in the opioid regimen significantly influence the GI microbiota as well as opioid dependence-related behaviors(43).

The endocannabinoid signaling is another pathway that the common genes significantly enriched in. In addition to be acknowledged to participate in the adipogenesis and obesity, the endocannabinoid system is currently under attention due to its potential therapeutic effects on a broad variety of disease including cancer(44). Drugs targeting the endocannabinoid system may have anti-metastatic effect to function against various types of tumors(45). Other functions of cannabinoid include anti-apoptotic, anti-angiogenic and anti-proliferative in the cancer cell growth through multiple pathways such as ERK, Akt, MAPK, PI3K and HIF-1(46). The endocannabinoid signaling is also reported to play a role in inflammation(47), energy balance(47), gastric barrier (48), adipogenesis(48), and neuroimmune disorder(49) by modulating the gastric microbiota. However, no evidence showed the whether the crosstalk between endocannabinoid signaling and gastric microbiota involve in gastric cancer or not up to now.

Apelin is a member of the ligand of its receptor called APJ (50). Both apelin and APJ are generally expressed in blood vascular and reported to play a role in the angiogenesis by promoting the growth of endothelial cells (51–53). Additional studies demonstrated that apelin is related to the maturation of tumor blood capillaries(54), tumor growth and lymph node metastasis in vivo (55). Wang et al. developed a series of experiments to examine the role of apelin in gastric cancer. The results showed that the tumor apelin expression status was associated with the clinical features of gastric cancer including tumor differentiation, lymph node and distant metastases. The further in vitro studies revealed that apelin played a role in modulating the migration and invasion abilities of gastric cancer cell lines(56). There is also evidence supports that GI microbiota is specifically related to the regulation of the apelinergic system in the type 2 diabetes(57). However, it is still remains to be determined whether apelin participate in the effect of GI microbiota on gastric carcinogenesis.

Conclusions:

Our data suggest that the GI microbiota might take part in the development of gastric cancer. The correlation between GI microbiota and gastric cancer involves a wide array of functions, interactions and pathways. The results of our study provide bioinformatics insights into the potential correlation of GI microbiota and gastric cancer. The specific roles of particular pathway need further discussion and investigation by means of examination and analysis.

Abbreviations

Gastric cancer

(GC)

Gastrointestinal

(GI)

Genome-wide association studies

(GWAS)

Human metabolome database

(HMDB)
Online Mendelian Inheritance in Man
(OMIM)
Search Tool for Interacting Chemicals
(STITCH)
WEB-based Gene SeT AnaLysis Toolkit
(WebGestalt)

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest:

No conflicts of interest declared.

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Authors' contributions:

XG designed the study. XLD, YKZ, ZYS, XYM carried out the bioinformatic analysis. XLD, YKZ, XWL, CCZ wrote the manuscript. Feng W, Fei W and XG reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Figures



Figure 1

Multiple bioinformatic approaches were used to analyze the potential interaction between gastrointestinal microbiota and gastric cancer. The bioinformatic analytic procedures are shown



Figure 2

Gene ontology analysis of 33 common genes shared by GI microbiota and GC conducted by WebGestalt is shown.



Figure 3

The 33 common genes shared by GI microbiota and GC were further analyzed by GeneMANIA, and the interaction networks were mapped by Cytoscape software.

Supplementary Files

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- [SupplementaryTable1.Pathwayanalysis.doc](#)