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Microbial and molecular differences according to the location of head and neck cancers

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Abstract

Background: Microbiome has been shown to substantially contribute to some cancers. However, the diagnostic implications of microbiome in head and neck squamous cell carcinoma (HNSCC) remain unknown.

Methods: To identify the molecular difference in the microbiome of oral and non-oral HNSCC, primary data was downloaded from the Kraken-TCGA dataset. The molecular differences in the microbiome of oral and non-oral HNSCC were identified using the linear discriminant analysis effect size method.

Results: In the study, the common microbiomes in oral and non-oral cancers were *Fusobacterium*, *Leptotrichia*, *Selemomonas* and *Treponema* and *Clostridium* and *Pseudoalteromonas*, respectively. We found unique microbial signatures that positively correlated with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in oral cancer and positively and negatively correlated KEGG pathways in non-oral cancer. In oral cancer, positively correlated genes were mostly found in prion diseases, Alzheimer disease, Parkinson disease, Salmonella infection, and Pathogenic Escherichia coli infection. In non-oral cancer, positively correlated genes showed Herpes simplex virus 1 infection and Spliceosome and negatively correlated genes showed results from PI3K-Akt signaling pathway, Focal adhesion, Regulation of actin cytoskeleton, ECM-receptor interaction and Dilated cardiomyopathy.

Conclusions: These results could help in understanding the underlying biological mechanisms of the microbiome of oral and non-oral HNSCC. Microbiome-based oncology diagnostic tool warrants further exploration.

Keywords: Microbiome, HNSCC, Oral cancer, Non-oral cancer, TCGA, KEGG pathway, Linear discriminant analysis

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, with 890,000 new cases and 450,000 deaths in 2018 [1, 2]. HNSCC accounts for about 6% of all cancers and 1–2% of deaths due to

neoplastic diseases [3–5]. HNSCC is a heterogeneous disease and tumours are distinguished based on location. HNSCC originates from the epithelial cells in the laryngeal and oropharynx, lips, mouth or larynx. Tobacco and alcohol consumption are the well-known and geographically most prevalent risk factors for HNSCC [6]. Heavy users of these carcinogens-containing products have a 35-fold higher risk of developing HNSCC than non-users [6, 7], and approximately three-quarters of HNSCC cases attributable to cigarette smoking and tobacco use [8]. In addition, betel nut chewing is independent risk factor for HNSCC in India, China or Taiwan [9, 10]. Especially,

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development of oropharyngeal cancers is strongly associated with HPV infection, which mainly occurs in Western Europe and the United States [6, 11].

Trillions of microbes have evolved and continue to live on and within human beings [12]. Numerous studies have suggested a link between the microbiota, which exist in various organs (e.g., gut and placenta) and pathological conditions such as neurologic diseases, metabolic disorders, and cancers [13–16]. With the development of omics technologies, such as metagenomics, transcriptomics, and proteomics, substantial evidence has been accumulated regarding the relationship of microorganisms and various diseases, including cancers [17].

The gut microbiome has been associated with various disorders, especially malignant tumours. The gut microbiome is involved in biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity [18]. Chronic diseases such as obesity, inflammatory bowel disease, diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease, non-alcoholic fatty liver disease, cirrhosis are associated with the human microbiome [19]. Several studies have demonstrated that gut microbiome dysbiosis is associated with tumourigenesis and/or tumour growth across cancer types, including colon, hepatocellular carcinoma, gastric, and breast [13, 18]. Moreover, the gut microbiome has been demonstrated to play a key role in the response to cancer therapy, such as chemotherapy, immune checkpoint blockade, and stem cell transplant [13]. For immune checkpoint blockade response, differential gut microbiome signatures exist in patients who respond to immune checkpoint blockade treatment [20–22].

Although intratumoral microbiota has not been studied as much as the gut microbiota, the importance of microbiota in tumours is increasing, with studies showing that it affects the response to cancer treatment [13, 23–26]. Intratumoral bacteria, which are metabolically active, can alter the chemical structure of anti-cancer drugs [27, 28]. In addition, *Fusobacterium nucleatum* in colorectal tumour promotes resistance to chemotherapy through modulation of autophagy [29]. HNSCC, especially oral squamous cell carcinoma (OSCC), is the most prevalent and commonly studied cancer associated with bacterial infection, and is the most common malignancy of the head and neck worldwide [30]. Two prominent oral pathogens, *Porphyromonas gingivalis*, and *F. nucleatum* have been reported to promote tumour progression in mice [31]. Periodontitis is an infectious disease causing chronic inflammation in the oral cavity [32, 33]. Periodontitis has been linked to various cancers, including oesophageal and oropharyngeal cancers [30]. Several studies have found that the risk of developing OSCC may

increase with periodontal disease [34, 35], and periodontal disease increases the risk of oral cancer even after adjusting for significant risk factors [36, 37]. Herein, we investigated the underlying molecular differences of the microbiome of oral cancer and non-oral HNSCC.

Methods

Microbiome datasets & TCGA RNA-sequencing datasets

We downloaded Kraken-TCGA(The Cancer Genome Atlas) -Raw-Data (n = 17,625) from microbial count datasets [38] for this study. Primary tumours were selected from HNSCC of microbiome data, classified into RNA and WGS, and combined with TCGA clinical information to separate oral and non-oral subtype. RNA-expression sequencing and clinical data sets of HNSCC samples were downloaded from the Broad GDAC Firehose [39] on 20 Feb 2020. The samples were categorised based on the site of occurrence as either oral cancer (alveolar ridge, buccal mucosa, floor of the mouth, hard palate, lip, oral cavity, and oral tongue) or non-oral cancer (base of tongue, hypopharyngeal, larynx, oropharynx, and tonsil) (Supplementary Table). Preprocessing was used with the R program (version 4.0.3) [40].

Linear discriminant analysis effect size (LEfSe)

To identify significantly different bacteria (as biomarkers) between the two groups at the genus level, taxa summaries were reformatted and inputted into LEfSe via the Huttenhower Lab Galaxy Server [41]. The LDA values of oral and non-oral HNSCC microbiome data of RNA and DNA were obtained. We used the LDA method to estimate the effect size of the abundant genus level [41].

Then, we obtained common bacteria of RNA and DNA with the threshold on the logarithmic LDA score for discriminative features of 2.0108 ($p < 0.0076$). In the settings of LEfSe, the Kruskal–Wallis sum-rank test ($\alpha = 0.05$) was used to detect taxa with significant differential abundance.

Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) and ANOVA-like differential expression (ALDEx2)

The name of the common bacteria was changed to ID of Greengenes (97% taxonomy) (version 13.5) (<http://greengenes.lbl.gov>) and used as an input file. PICRUSt was performed using the Galaxy web application, which was used to predict bacterial metabolic contributions of oral rich and non-oral rich bacteria, respectively [42]. To filter the results of the PICRUSts, we merged results of oral rich and non-oral rich bacteria, and used the ALDEx2 [43] to obtain top five pathways with a p-value of 0.05 or less.

Correlation analysis

A correlation analysis was performed with respect to the RNA expression data and common bacteria data of oral and non-oral HNSCC. Using the Spearman correlation test, genes with oral/non-oral correlation coefficients $r > 0.15$ and $r < -0.15$ were obtained. Significance levels were considered at $P < 0.05$.

Protein–protein interaction (PPI) analysis & Hub gene

PPI analysis of correlated genes was performed using the plug-in Search Tool for the Retrieval of Interacting Genes (STRING) app (version 1.5.1) [44]. The results of the analysis were imported into Cytoscape (version 3.8.2) [45] to establish a network model. The plug-in app cytohubba (version 0.1) [46] in Cytoscape was downloaded and installed. The top ten scores of the degree algorithm were taken as the criteria to screen out the hub genes with high connectivity in the gene expression network.

KEGG pathway and gene ontology (GO)

KEGG pathway and GO analysis were performed on the DAVID website [47] with the genes in the node table resulting from the PPI. Then, the genetic symbol was transferred to entrezID using the org.Hs.eg.db (version 3.12.0) package [48] with the same input file from the PPI for subsequent analysis. The results of enhanced GO entries and KEGG were visualised as path point plots using clusterProfiler (version 3.18.1), ggplot (version 3.3.5), and Enrichplot2 (version 1.10.2) packages. GO and KEGG analysed the used data with statistically significant false discovery rates < 0.05 .

Results

Characterisation of unique microbial signatures of oral and non-oral HNSCC

To evaluate the unique microbial signatures of oral and non-oral HNSCC, we analysed Kraken-TCGA data sets using the linear discriminant analysis (LDA) method. We divided 691 HNSCC samples into 172 DNA whole genome sequencing (WGS) data and 519 RNA sequencing data (Fig. 1). Next, we analysed RNA sequencing as subtypes divided into 314 oral cancer and 205 non-oral cancer. DNA WGS data were also analysed as 115 oral and 57 non-oral subtypes. Clinical information related to these samples is described in Table 1. In both data, gender ($P = 8.698E-05$ (RNA)/ $2.372E-06$ (DNA)) HPV status ($P = 1.623E-09$ (RNA)/ $5.201E-08$ (DNA)), clinical stage ($P = 3.998E-03$ (RNA)/ $1.100E-03$ (DNA)) and pathologic stage ($P = 4.998E-04$ (RNA)/ $2733E-05$ (DNA)) were significantly different between patients with oral and non-oral cancers.

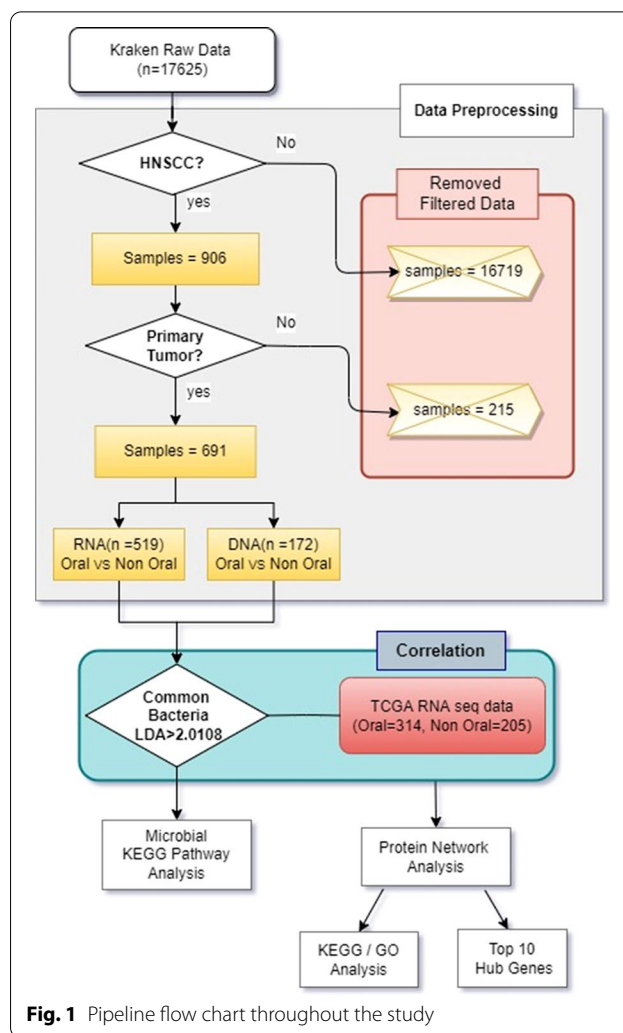


Fig. 1 Pipeline flow chart throughout the study

Investigation of the common microbiome of oral and non-oral HNSCC

The relatively enriched microbiome of oral and non-oral HNSCC are shown in Fig. 2a, b. The enriched microbiomes in oral HNSCC were *Fusobacterium*, *Leptotrichia*, *Seelenomonas* and *Treponema* and the enriched microbiomes in non-oral HNSCC were *Clostridium* and *Pseudoalteromonas*, as determined by the linear discriminant analysis effect size (LEfSe) method (Fig. 2a, b). The distribution of count data for each microbiome subtypes is depicted in Fig. 2c–h.

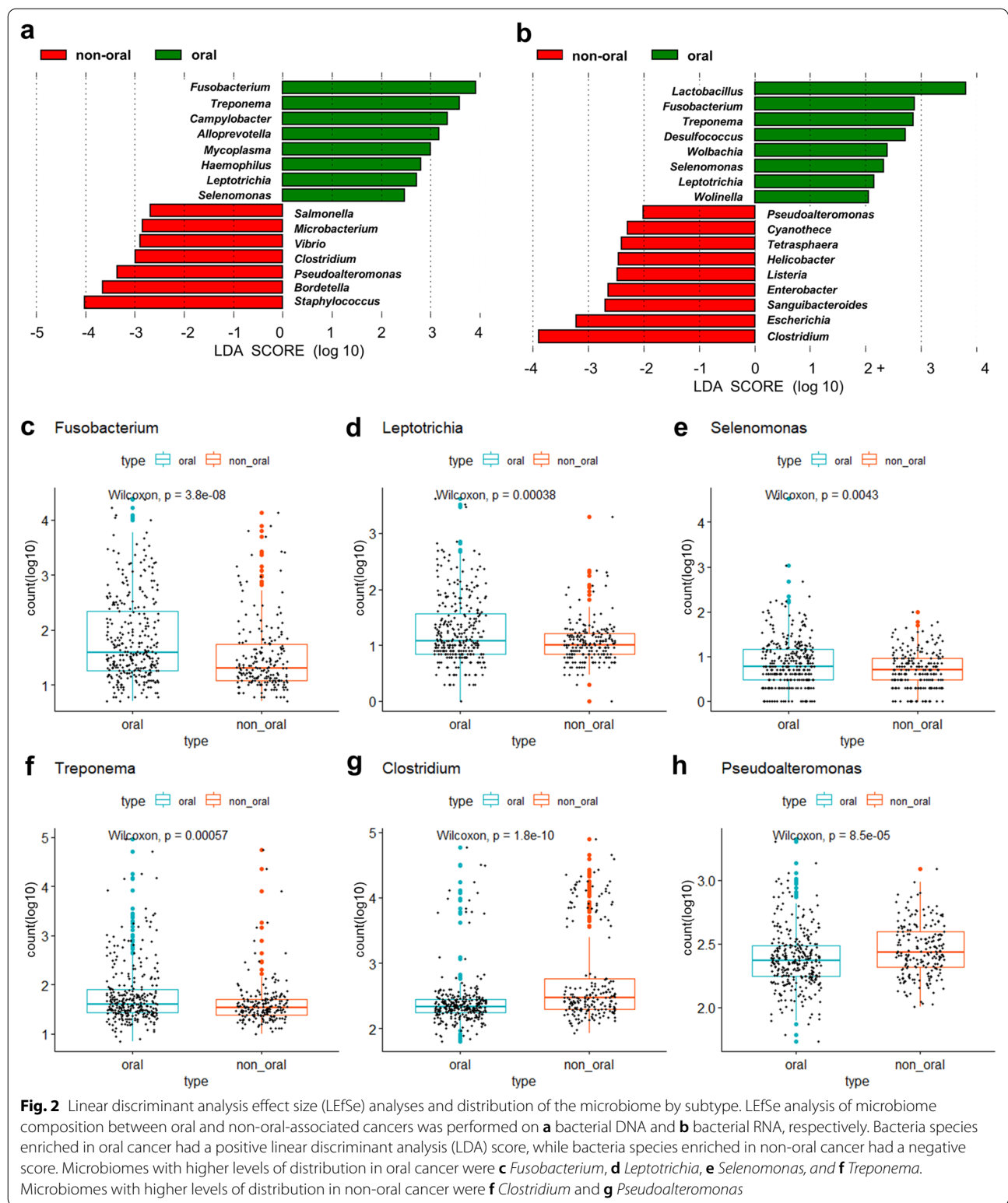
Microbial Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and protein network of oral and non-oral HNSCC

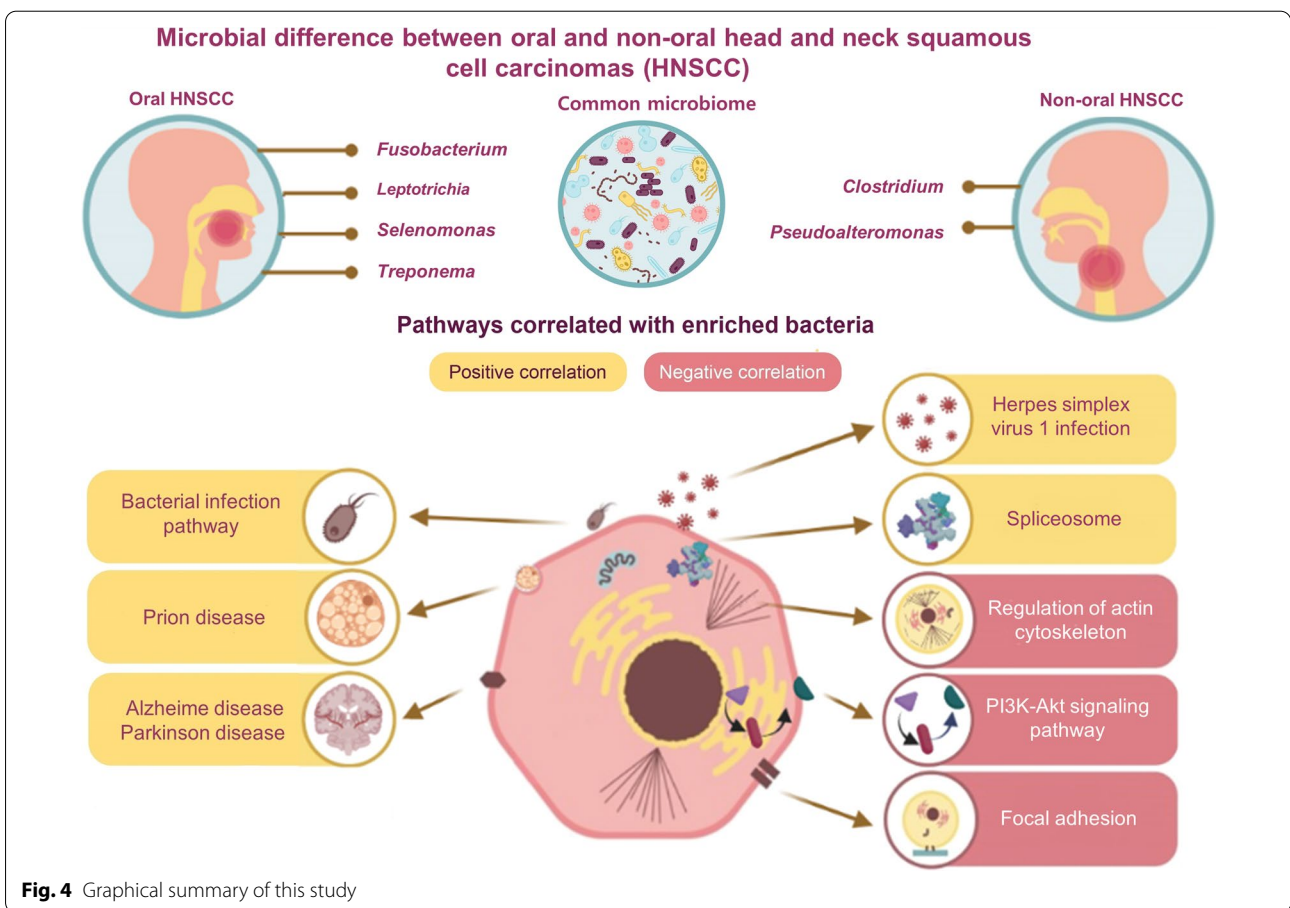
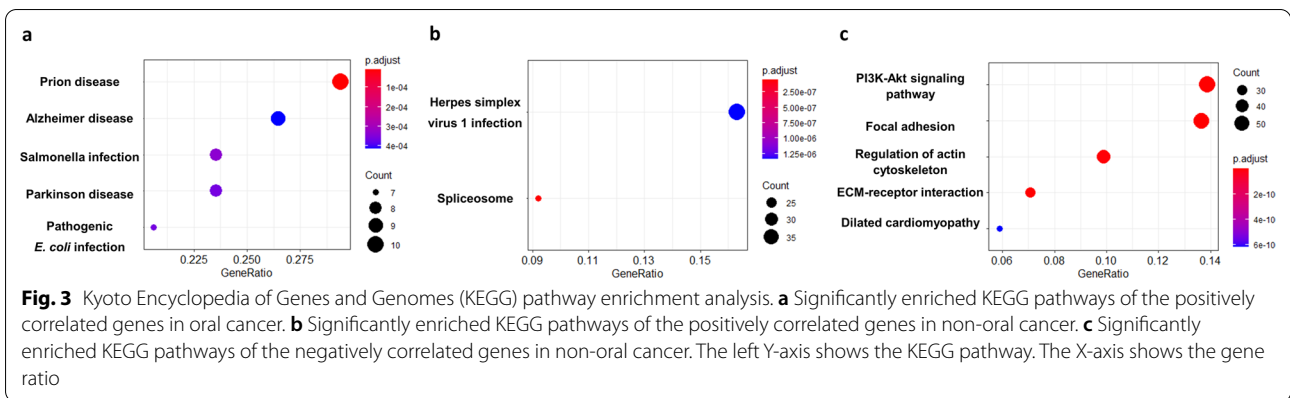
We analysed the molecular mechanism of the microbiome of oral and non-oral HNSCC using KEGG pathway analysis and protein network analysis (Fig. 3, Tables 1 and

Table 1 Patient's characteristics

Variables	RNA (N = 519)			DNA (N = 172)			
	Oral (314)	Non-oral (205)	P-value	Oral (115)	Non-oral (57)	P-value	
Age	<66	202 (64%)	154 (75%)	0.030*	80 (70%)	43 (75%)	0.476
	≥66	111 (35%)	51 (25%)		35 (30%)	14 (25%)	
Gender	NA	1 (0%)	–		–	–	–
	Female	102 (32%)	34 (17%)	8.698E-05***	41 (36%)	–	2.372E-06***
HPV status	Male	212 (68%)	171 (83%)		74 (64%)	51 (89%)	
	positive	32 (10%)	65 (32%)	1.623E-09***	16 (14%)	31 (54%)	5.201E-08***
Clinical Stage	negative	282 (90%)	140 (68%)		99 (86%)	26 (46%)	
	NA	1 (0%)	–		–	–	–
Pathologic Stage	Stage I	12 (4%)	8 (4%)	3.998E-03**	4 (3%)	–	1.100E-03***
	Stage II	76 (24%)	22 (11%)	4.998E-04***	29 (25%)	9 (16%)	
Race	Stage III	65 (21%)	40 (20%)		29 (25%)	7 (12%)	
	Stage IVA	146 (46%)	118 (58%)		53 (46%)	35 (61%)	
Alcohol History	Stage IVB	4 (1%)	7 (3%)		–	4 (7%)	
	Stage IVC	3 (1%)	4 (2%)		–	1 (2%)	
Pack Years Smoked	NA	8 (3%)	6 (3%)		–	1 (2%)	
	Stage I	21 (7%)	6 (3%)		9 (8%)	2 (4%)	2.733E-05***
Pathologic Stage	Stage II	54 (17%)	20 (10%)		22 (19%)	4 (7%)	
	Stage III	56 (18%)	25 (12%)		18 (16%)	8 (14%)	
Race	Stage IVA	154 (49%)	98 (48%)		56 (49%)	19 (33%)	
	Stage IVB	7 (2%)	5 (2%)		1 (1%)	2 (4%)	
Alcohol History	Stage IVC	–	1 (0%)		–	–	
	NA	22 (7%)	50 (24%)		9 (8%)	22 (39%)	
Pack Years Smoked	American Indian or Alaska native	1 (0%)	1 (0%)	0.029*	–	–	0.379
	Asian	10 (3%)	1 (0%)		2 (2%)	–	
Pathologic Stage	Black or African American	22 (7%)	26 (13%)		6 (5%)	6 (11%)	
	White	270 (86%)	173 (84%)		105 (91%)	51 (89%)	
Alcohol History	NA	11 (4%)	4 (2%)		2 (2%)	–	
	Yes	202 (64%)	144 (70%)	0.393	72 (63%)	48 (84%)	1.913E-03**
Pack Years Smoked	NO	105 (33%)	57 (28%)		41 (36%)	7 (12%)	
	NA	7 (2%)	4 (2%)		2 (2%)	2 (4%)	
Pathologic Stage	30 <	52 (17%)	37 (18%)	0.014*	16 (14%)	12 (21%)	0.174
	30 ≥	111 (35%)	95 (46%)		42 (37%)	25 (44%)	
Race	NA	151 (48%)	73 (36%)		57 (50%)	20 (35%)	
	American Indian or Alaska native	–	–		–	–	
Alcohol History	Asian	–	–		–	–	
	Black or African American	–	–		–	–	
Pack Years Smoked	White	–	–		–	–	
	NA	–	–		–	–	
Pathologic Stage	Yes	–	–		–	–	
	NO	–	–		–	–	
Race	30 <	–	–		–	–	
	30 ≥	–	–		–	–	
Alcohol History	NA	–	–		–	–	
	Yes	–	–		–	–	

AJCC version:4–7th, P < 0.05 ** P < 0.01 *** P < 0.001, HNSCC, head and neck squamous cell carcinoma; NA not available
 Chi-squared test was done for gender, HPV status, Pack Years Smoked and Fisher's exact-test was done for Age, Clinical Stage, Pathologic Stage, Race, Alcohol History





2). We found unique microbial signatures that positively correlated KEGG pathways in oral HNSCC, positively correlated KEGG pathways and negatively correlated KEGG pathways in non-oral HNSCC (Figs. 3 and 4). In oral HNSCC, positively correlated genes were mostly found in bacterial infection pathways, and the genes involved in neurodegenerative diseases (prion diseases, Alzheimer disease, and Parkinson disease). In non-oral cancer, positively correlated genes were found Herpes

simplex virus 1 infection and Spliceosome and negatively correlated genes showed results from PI3K-Akt signaling pathway, focal adhesion and regulation of actin cytoskeleton and Dilated cardiomyopathy. In addition, we conducted a pathway and gene expression analysis using microbial data of subtypes from each oral and non-oral HNSCC. As a result of PICRUST, rich microbiome within oral cancer was involved in germination, Huntington’s disease, biosynthesis of siderophore group nonribosomal

Table 2 Results of PICRUSt KEGG pathway enrichment analysis

	Level 1	Level 2	Level 3	Rab.win. non-oral	Rab.win.oral	diff.btw
Oral rich bacteria	Unclassified	Cellular Processes and Signaling	Germination	− 0.421	8.339	9.005
	Human diseases	Neurodegenerative diseases	Huntington's disease	0.541	8.378	7.972
	Metabolism	Metabolism of Terpenoids and Polyketides	Biosynthesis of siderophore group nonribosomal peptides	1.232	8.327	7.111
	Metabolism	Xenobiotics Biodegradation and Metabolism	Atrazine degradation	1.559	8.333	6.271
	Human diseases	Neurodegenerative diseases	Prion diseases	2.353	8.343	6.171
Non-oral rich bacteria	Metabolism	Glycan biosynthesis and metabolism	Other glycan degradation	10.295	− 0.612	− 10.855
	Cellular processes	Transport and catabolism	Lysosome	10.270	− 0.458	− 10.537
	Metabolism	Glycan biosynthesis and metabolism	Glycosphingolipid biosynthesis—globo series	10.230	0.474	− 9.590
	Unclassified	Cellular processes and signaling	Electron transfer carriers	10.290	1.406	− 8.713
	Metabolism	Glycan biosynthesis and metabolism	Glycosaminoglycan degradation	10.275	1.553	− 8.588

BH < 0.05 compared to the oral and non-oral (ALDEx2); BH Benjamini-Hochberg

diff.btw cut off > abs(6)

rab.win.non-oral: a vector containing the median clr value for each feature in non-oral, clr centred log-ratio

rab.win.oral: a vector containing the median clr value for each feature in oral

diff.btw: a vector containing the per-feature median difference between condition non-oral and oral

PICRUSt phylogenetic investigation of communities by reconstruction of unobserved states; KEGG Kyoto Encyclopedia of Genes and Genomes

peptides, atrazine degradation and prion diseases. Rich microbiome within non-oral cancer was found to be associated with other glycan degradation, Lysosome, Glycosphingolipid biosynthesis—globo series, electron transfer carriers, and glycosaminoglycan degradation (Table 2 and Additional file 2: Table S1). Rich microbiome within non-oral cancer was found to be associated with biosynthesis and metabolism of glycan, transport, catabolism, and biosynthesis of other secondary metabolites. Rich microbiome within oral cancer was involved in the biodegradation and metabolism of xenobiotics, neurodegenerative diseases, and the circulatory system. We found significant pathways using correlated genes with microbiome. We identified the KEGG pathways by selecting only the noded genes as a protein–protein interaction tool (Table 3). The results of the phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis are shown in Additional file 1: Fig. S1. ALDEx2 was performed by merging the KEGG pathways obtained after PICRUSt of each subtype. The result is the median expression value of the KEGG pathway, and is expressed as a dot on the graph (Additional file 2: Table S1).

Discussion

The microbiome plays an important role in the human host and participates in the development of a wide variety of diseases, such as cancer [12]. The tumor microbiome is associated with a chronic inflammatory state and modulates the initiation and development of various cancers, such as lung, breast, colon, gastric, pancreatic, cholangiocarcinoma, ovarian, and prostate cancers [13, 23–26, 49–51]. In colorectal cancer (CRC), transplant of stool containing the tumor microbiome from patients with CRC can induce polyp formation [52, 53]. Moreover, some bacterial species (*F. nucleatum*) can stimulate an inflammatory state that can promote carcinogenesis via increased production of reactive oxygen species [54], induction of proinflammatory toxins [55, 56], and suppression of anti-tumor immune functions [57, 58]. In this study, for the first time, we differentiated the microbiota of HNSCC into oral and non-oral cancers to identify differences in the abundance of the tumor microbiome. Then, we then attempted a molecular approach using the correlation between the microbiome and mRNA expression. We systematically selected six microbiomes as unique microbial signatures of oral and non-oral

Table 3 DAVID gene-annotation enrichment analysis of KEGG pathway

	ID	KEGG pathway	Count	P-value	FDR	Genes
Positively correlated genes in oral cancer	hsa05020	Prion disease	10	9.21E-07	9.120E-05	STIP1, PSMA6, TUBA1C, PSMD12, TUBB6, TUBB2A, IL1B, PPIF, TUBB4B, TUBA4A
	hsa05010	Alzheimer disease	9	1.15E-04	2.412E-03	PSMA6, TUBA1C, PSMD12, TUBB6, TUBB2A, IL1B, PPIF, TUBB4B, TUBA4A
	hsa05132	Salmonella infection	8	5.09E-05	2.412E-03	TUBA1C, TUBB6, TUBB2A, CXCL8, IL1B, TUBB4B, DYNLL1, TUBA4A
	hsa05012	Parkinson disease	8	7.74E-05	2.412E-03	PSMA6, TUBA1C, PSMD12, TUBB6, TUBB2A, PPIF, TUBB4B, TUBA4A
	hsa05130	Pathogenic Escherichia coli infection	7	1.22E-04	2.412E-03	TUBA1C, TUBB6, TUBB2A, CXCL8, IL1B, TUBB4B, TUBA4A
Positively correlated genes in oral cancer	hsa05168	Herpes simplex virus 1 infection	39	6.31067961	3.342E-08	ZNF155, ZNF132, ZNF550, ZNF195, ZNF606, ZNF84, ZNF823, ZNF547, ZNF205, ZNF766, ZNF600, ZNF226, ZNF302, EIF2B1, ZNF566, ZNF620, ZNF224, ZNF564, ZNF443, ZNF584, ZNF441, ZNF141, ZNF140, ZNF283, BST2, IRF3, ZNF519, IRF7, SRSF2, SRSF3, ZNF337, ZNF557, SRSF5, ZNF780A, SRSF6, SRSF7, ZNF112, ZNF530, ZNF354B
	hsa03040	Spliceosome	22	3.55987055	1.454E-09	PRPF38B, HSPA1L, RBM8A, CCDC12, THOC1, MAGOHB, LSMS, LSM4, LSM2, XAB2, HNRNP, PHF5A, PRPF18, TRA2B, MAGOH, SRSF2, SRSF3, PRPF31, SRSF5, SRSF6, SRSF7, SRSF10
Negatively correlated genes in non-oral cancer	hsa04151	PI3K-Akt signaling pathway	59	6.5701559	1.870E-15	ITGB1, ATF2, FLT1, ITGB5, IRS1, ITGB4, FLT4, ITGB3, TNC, LAMC2, LAMC1, IGF1R, RPTOR, GYS1, PPP2R5E, CREB3L2, KDR, ITGAV, ITGB6, IL6R, YWHAG, PDGFRB, MAP2K1, ITGA3, ITGA1, F2R, PRKCA, OSMR, COL4A2, PIK3CA, COL4A1, COL6A1, COL6A3, ITGA7, ITGA6, ITGA5, ITGA9, CREB5, LAMA2, LAMA4, LAMA3, PDGFB, LPAR3, LPAR4, THBS2, THBS1, EGFR, REL, RXRA, PDGFC, MAPK1, ANGPT2, LAMB3, FN1, PPP2R3A, COL1A1, COL1A2, ITGA11, TEK
	hsa04510	Focal adhesion	58	6.45879733	1.455E-27	ITGB1, FLT1, ITGB5, ITGB4, FLT4, ITGB3, TNC, LAMC2, LAMC1, ACTB, IGF1R, MYLK, KDR, ITGAV, ITGB6, PDGFRB, MAP2K1, ITGA3, ACTN1, ITGA1, PRKCA, ACTN4, COL4A2, PIK3CA, COL4A1, COL6A1, RAPGEF1, COL6A3, ITGA7, ITGA6, ITGA5, TLN1, CRK, VCL, ITGA9, LAMA2, ROCK2, LAMA4, PXN, LAMA3, PDGFB, THBS2, THBS1, EGFR, PDGFC, FLNA, MAPK1, FLNB, FLNC, PAK2, LAMB3, CAV1, FN1, PARVA, COL1A1, COL1A2, ITGA11, ZYX
	hsa04810	Regulation of actin cytoskeleton	42	4.67706013	3.317E-13	ITGB1, CYFIP1, ITGB5, ROCK2, ITGB4, ITGB3, ARPC1B, PXN, PDGFB, WASL, LPAR4, IQGAP1, EGFR, ACTB, SLC9A1, MYLK, GNA12, PDGFC, MAPK1, ITGAV, ITGB6, PAK2, PDGFRB, MAP2K1, ITGA3, ACTN1, LIMK1, ITGA1, F2R, FN1, MSN, ACTN4, ENAH, PIK3CA, ITGA11, MYH9, ITGA7, ITGA6, ITGA5, CRK, VCL, ITGA9
	hsa04512	ECM-receptor interaction	30	3.34075724	1.945E-16	ITGB1, LAMA2, ITGB5, ITGB4, LAMA4, ITGB3, LAMA3, TNC, LAMC2, LAMC1, THBS2, THBS1, ITGAV, ITGB6, LAMB3, ITGA3, ITGA1, FN1, HSPG2, COL1A1, COL1A2, COL4A2, COL4A1, COL6A1, ITGA11, COL6A3, ITGA7, ITGA6, ITGA5, ITGA9
	hsa05414	Dilated cardiomyopathy	25	2.78396437	6.870E-11	ITGB1, LAMA2, ITGB5, ITGB4, ITGB3, ATP2A2, ADCY1, ADCY7, ACTB, SGCD, SGCA, ITGAV, ITGB6, TPM4, ITGA3, TPM1, ITGA1, ACTC1, DES, ITGA11, MYL3, ITGA7, ITGA6, ITGA5, ITGA9

FDR false discovery rate

HNSCC. Microbiomes with higher levels of distribution in oral HNSCC were *Selenomonas*, *Fusobacterium*, *Leptotrichia* and *Treponema*, while microbiomes with higher levels of distribution in non-oral HNSC were *Clostridium* and *Pseudoalteromonas*.

The relationship between oral microbiota and human diseases has studied a lot. Especially, several bacteria including *Porphyromonas gingivalis*, *Treponema denticola*, *Selenomonas sputigena* and *Fusobacterium nucleatum* have been associated with cancer development [59–61]. In the current study, we observed the *Fusobacterium*, *Treponema*, *Leptotrichia* were enriched in oral cancer compared to non-oral cancer. In consistent with previous research, it may have a negative effect on cancer progression. *Clostridium* species, which are well-studied anaerobic bacterium, has high ability for colonization in the hypoxic and necrotic lesions in tumour [62]. Genetically modified *Clostridium* expressing tumour suppressive genes is one of the therapeutic strategies of cancers. Since the *Clostridium* is enriched in non-oral cancer, it may be used as therapeutic options for non-oral cancers.

The prevention and treatment of diseases by targeting the microbiome have been widely investigated [30]. Modulation of the microbiome may also contribute to the treatment of cancer [63]. Cancer therapy requires an intact commensal microbiome that mediates the therapy effects by modulating functions of myeloid-derived suppressor cells in the tumor microenvironment [24, 63, 64]. Some studies have shown the deleterious effects of antibiotics on the treatment of cancer [13, 65]. Patients with metastatic renal cell carcinoma or non-small-cell lung cancer had significantly worse survival outcomes if they received antibiotics just before or just after the initiation of treatment with immune checkpoint blockade [66]. In addition, patients who received anti-Gram-positive antibiotics along with cyclophosphamide for chronic lymphocytic leukemia or cisplatin for relapsed lymphoma had a lower overall response rate [55, 67]. These microbiomes may confer susceptibility to certain cancers, either through a direct effect by the local presence within the tumor microenvironment or via the systemic impact of the microbiome from a distant location, such as the gut and the skin [68].

There are several limitations in this study. The results were not validated in other cohorts or experimental procedures. We obtained the results by using Kraken pipeline, which obtains microbiome information from whole genome sequencing or RNA sequencing data. Therefore, it is necessary to verify it by microbiome sequencing and/or PCR analysis.

Taken together, stress conditions, such as diet, antigen exposure, medications, and stress are important factors that contributing to the state of health and also

affect the microbiome [38]. This field is young, and we are left with many unanswered questions—especially regarding the mechanism of action as well as the group of bacterial species that are most important in mediating antitumor effects. Multifaceted strategies are needed to modulate precision medicine and treat disease. Efforts are currently underway to enhance therapeutic responses and/or abrogate treatment-associated toxicity chemotherapeutic agents via modulation of the microbiome.

Abbreviations

TCGA: The Cancer Genome Atlas; ALDEx2: ANOVA-like differential expression tool for high-throughput sequencing data; CRC: Colorectal cancer; GO: Gene Ontology; HNSCC: Head and neck squamous cell carcinoma; KEGG: Kyoto Encyclopedia of Genes and Genomes; LDA: Linear discriminant analysis; LEfSe: Linear discriminant analysis effect size; OSCC: Oral squamous cell carcinoma; PICRUSt: Phylogenetic investigation of communities by reconstruction of unobserved states; PPI: Protein–protein interaction; STRING: Search Tool for the Retrieval of Interacting Genes/Proteins; TCGA: The Cancer Genome Atlas; WGS: Whole Genome sequencing.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12935-022-02554-6>.

Additional file 1: Figure S1. Output from ALDEx2 plot.

Additional file 2: Table S1. The GO analysis results, hub genes, and tumour locations of included patients.

Acknowledgements

Not applicable.

Authors' contributions

DL and YHK initiated the study and guided the work. YKK and EJK collected, normalised, and interpreted the data. YY, JK, SYW, HSC, MK, KJ, HSK, and HRP analysed the experimental data. All authors wrote the manuscript with input from all co-authors. All authors read and approved the final manuscript.

Funding

This work was supported by the National Research Foundation of Korea (NRF-2018R1A5A2023879, 2020R1A2C1005203, 2020R1C1C1003741, and 2021R1A2C4001466). This research was supported by a grant of the Medical data-driven hospital support project through the Korea Health Information Service (KHIS), funded by the Ministry of Health & Welfare, Republic of Korea. A portion of the data used for this study were obtained from the Genome-InfraNet (IDs: 1711020733, 1711032008, and 1711028992) of the Korea Bioinformatics Center.

Availability of data and materials

All the data were available on the manuscript or from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed on the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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Received: 3 January 2022 Accepted: 15 March 2022

Published online: 26 March 2022

References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394.
- Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *Int J Cancer*. 2019;144:1941.
- Attar E, Dey S, Hablas A, Seifeldin IA, Ramadan M, Rozek LS, et al. Head and neck cancer in a developing country: a population-based perspective across 8 years. *Oral Oncol*. 2010;46:591.
- Nandakumar A. Survival in head and neck cancers—results of a multi-institution study. *Asian Pac J Cancer Prev*. 2016;17:1745.
- Pai SI, Westra WH. Molecular pathology of head and neck cancer: implications for diagnosis, prognosis, and treatment. *Annu Rev Pathol*. 2009;4:49.
- Johnson DE, Burtneis B, Leemans CR, Lui WY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. *Nat Rev Dis Prim*. 2020. <https://doi.org/10.1038/s41572-020-00224-3>.
- Hashibe M, Brennan P, Chuang S-C, Boccia S, Castellsague X, Chen C, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the international head and neck cancer epidemiology consortium. *Cancer Epidemiol Biomark Prev*. 2009;18:541.
- Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, et al. Smoking and drinking in relation to oral and pharyngeal cancer. *Can Res*. 1988;48:3282.
- Lin YS, Jen YM, Wang BB, Lee JC, Kang BH. Epidemiology of oral cavity cancer in taiwan with emphasis on the role of betel nut chewing. *ORL*. 2005;67:230.
- Su YY, Chien CY, Luo SD, Huang TL, Lin WC, Fang FM, et al. Betel nut chewing history is an independent prognosticator for smoking patients with locally advanced stage IV head and neck squamous cell carcinoma receiving induction chemotherapy with docetaxel, cisplatin, and fluorouracil. *World J Surg Oncol*. 2016;14:86.
- Wang C-P, Chen T-C, Chen H-H, Hsu W-L, Chang Y-L. Prevalence of current oral HPV infection among 100 betel nut chewers or cigarette smokers in Northern Taiwan. *J Formos Med Assoc*. 2019;118:203.
- Koh A, Bäckhed F. From association to causality: the role of the gut microbiota and its functional products on host metabolism. *Mol Cell*. 2020;78:584.
- Helmink BA, Khan MW, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. *Nat Med*. 2019;25:377.
- Czesnikiewicz-Guzik M, Müller DN. Scientists on the spot: salt, the microbiome, and cardiovascular diseases. *Cardiovasc Res*. 2018;114:e72.
- Hansen JJ, Sartor RB. Therapeutic manipulation of the microbiome in IBD: current results and future approaches. *Curr Treat Options Gastro*. 2015;13:105.
- Kim D, Zeng MY, Núñez G. The interplay between host immune cells and gut microbiota in chronic inflammatory diseases. *Exp Mol Med*. 2017;49:e339.
- Salihoglu R, Onal-Süzek T. Tissue Microbiome Associated With Human Diseases by Whole Transcriptome Sequencing and 16S Metagenomics. *Front Genet*. 2021;12:281.
- Papotto PH, Yilmaz B, Silva-Santos B. Crosstalk between $\gamma\delta$ T cells and the microbiota. *Nat Microbiol*. 2021. <https://doi.org/10.1038/s41564-021-00948-2>.
- Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human gut microbes associated with obesity. *Nature*. 2006;444:1022.
- Routy B, Le Chatelier E, Derosa L, Duong CP, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. *Science*. 2018;359:91.
- Matson V, Fessler J, Bao R, Chongsuwan T, Zha Y, Alegre M-L, et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*. 2018;359:104.
- Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews M, Karpinetz T, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*. 2018;359:97.
- Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, Costa JL, Carneiro F, Machado JC, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. *Gut*. 2018;67:226.
- Pushalkar S, Hundeyin M, Daley D, Zambirinis CP, Kurz E, Mishra A, et al. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. *Cancer Discov*. 2018;8:403.
- Sfanos KS, Sauvageot J, Fedor HL, Dick JD, De Marzo AM, Isaacs WB. A molecular analysis of prokaryotic and viral DNA sequences in prostate tissue from patients with prostate cancer indicates the presence of multiple and diverse microorganisms. *Prostate*. 2008;68:306.
- Urbaniak C, Gloor GB, Brackstone M, Scott L, Tangney M, Reid G. The microbiota of breast tissue and its association with breast cancer. *Appl Environ Microbiol*. 2016;82:5039.
- Lehouritis P, Cummins J, Stanton M, Murphy CT, McCarthy FO, Reid G, et al. Local bacteria affect the efficacy of chemotherapeutic drugs. *Sci Rep*. 2015;5:1.
- Panebianco C, Andriulli A, Paziienza V. Pharmacomicrobiomics: exploiting the drug-microbiota interactions in anticancer therapies. *Microbiome*. 2018;6:1.
- Yu T, Guo F, Yu Y, Sun T, Ma D, Han J, et al. *Fusobacterium nucleatum* promotes chemoresistance to colorectal cancer by modulating autophagy. *Cell*. 2017;170:548.
- Irfan M, Delgado RZR, Frias-Lopez J. The oral microbiome and cancer. *Front Immunol*. 2020. <https://doi.org/10.3389/fimmu.2020.591088>.
- Gallimidi AB, Fischman S, Revach B, Bulvik R, Maliutina A, Rubinstein AM, et al. Periodontal pathogens *Porphyromonas gingivalis* and *Fusobacterium nucleatum* promote tumor progression in an oral-specific chemical carcinogenesis model. *Oncotarget*. 2015;6:22613.
- Hajishengallis G. Immunomicrobial pathogenesis of periodontitis: key-stones, pathobionts, and host response. *Trends Immunol*. 2014;35:3.
- Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. *Nat Rev Immunol*. 2015;15:30.
- Galvão-Moreira LV, da Cruz MCFN. Oral microbiome, periodontitis and risk of head and neck cancer. *Oral Oncol*. 2016;53:17.
- Karmakar S, Kar A, Thakur S, Rao VU. Periodontitis and oral Cancer-A striking link. *Oral Oncol*. 2020;106:104630.
- Fitzpatrick SG, Katz J. The association between periodontal disease and cancer: a review of the literature. *J Dent*. 2010;38:83.
- Javed F, Warnakulasuriya S. Is there a relationship between periodontal disease and oral cancer? A systematic review of currently available evidence. *Crit Rev Oncol Hematol*. 2016;97:197.
- Poore GD, Kopylova E, Zhu Q, Carpenter C, Fraraccio S, Wandro S, et al. Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nature*. 2020;579:567.
- Deng M, Brägelmann J, Kryukov I, Saraiva-Agostinho N, Perner S. Firebrowser: an R client to the Broad Institute's Firehose Pipeline. Database. 2017. <https://doi.org/10.1093/database/baw160>.
- Team RC. R: A language and environment for statistical computing ,Vienna. 2013.<http://www.R-project.org/>

41. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. *Genome Biol.* 2011;12:R60.
42. Langille MGJ, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol.* 2013;31:814.
43. Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome.* 2014;2:15.
44. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 2019;47:D607.
45. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ. Cytoscape StringApp: network analysis and visualization of proteomics data. *J Proteome Res.* 2019;18:623.
46. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. *BMC Syst Biol.* 2014;8(Suppl 4):S11.
47. Jiao X, Sherman BT, Huang DW, Stephens R, Baseler MW, Lane HC, et al. DAVID-WS: a stateful web service to facilitate gene/protein list analysis. *Bioinformatics.* 2012;28:1805.
48. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS.* 2012;16:284.
49. Banerjee S, Tian T, Wei Z, Shih N, Feldman MD, Alwine JC, et al. The ovarian cancer oncobiome. *Oncotarget.* 2017;8:36225.
50. Mao Q, Jiang F, Yin R, Wang J, Xia W, Dong G, et al. Interplay between the lung microbiome and lung cancer. *Cancer Lett.* 2018;415:40.
51. Aviles-Jimenez F, Guitron A, Segura-Lopez F, Mendez-Tenorio A, Iwai S, Hernandez-Guerrero A, et al. Microbiota studies in the bile duct strongly suggest a role for *Helicobacter pylori* in extrahepatic cholangiocarcinoma. *Clin Microbiol Infect.* 2016;22:178.e11.
52. Nakatsu G, Li X, Zhou H, Sheng J, Wong SH, Wu WKK, et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. *Nat Commun.* 2015;6:8727.
53. Lu Y, Chen J, Zheng J, Hu G, Wang J, Huang C, et al. Mucosal adherent bacterial dysbiosis in patients with colorectal adenomas. *Sci Rep.* 2016;6:26337.
54. Mangerich A, Knutson CG, Parry NM, Muthupalani S, Ye W, Prestwich E, et al. Infection-induced colitis in mice causes dynamic and tissue-specific changes in stress response and DNA damage leading to colon cancer. *Proc Natl Acad Sci.* 2012;109:E1820.
55. Wu S, Rhee K-J, Albesiano E, Rabizadeh S, Wu X, Yen H-R, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med.* 2009;15:1016.
56. Boleij A, Hechenbleikner EM, Goodwin AC, Badani R, Stein EM, Lazarev MG, et al. The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin Infect Dis.* 2015;60:208.
57. Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, et al. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity.* 2015;42:344.
58. Arthur JC, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan T-J, et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science.* 2012;338:120.
59. Tuominen H, Rautava J. Oral Microbiota and Cancer Development. *Pathobiology.* 2021;88:116.
60. Nieminen MT, Listyarifah D, Hagstrom J, Haglund C, Grenier D, Nordstrom D, et al. *Treponema denticola* chymotrypsin-like proteinase may contribute to orodigestive carcinogenesis through immunomodulation. *Br J Cancer.* 2018;118:428.
61. Rodriguez RM, Hernandez BY, Menor M, Deng Y, Khadka VS. The landscape of bacterial presence in tumor and adjacent normal tissue across 9 major cancer types using TCGA exome sequencing. *Comput Struct Biotechnol J.* 2020;18:631.
62. Theys J, Lambin P. Clostridium to treat cancer: dream or reality? *Ann Transl Med.* 2015;3:S21.
63. Iida N, Dzutsev A, Stewart CA, Smith L, Bouladoux N, Weingarten RA, et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science.* 2013;342:967.
64. Orberg ET, Fan H, Tam AJ, Dejea CM, Shields CD, Wu S, et al. The myeloid immune signature of enterotoxigenic *Bacteroides fragilis*-induced murine colon tumorigenesis. *Mucosal Immunol.* 2017;10:421.
65. Arthur JC, Gharaibeh RZ, Uronis JM, Perez-Chanona E, Sha W, Tomkovich S, et al. VSL# 3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. *Sci Rep.* 2013;3:1.
66. Derosa L, Hellmann M, Spaziano M, Halpenny D, Fidelle M, Rizvi H, et al. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. *Ann Oncol.* 2018;29:1437.
67. Pflug N, Kluth S, Vehreschild JJ, Bahlo J, Tacke D, Biehl L, et al. Efficacy of antineoplastic treatment is associated with the use of antibiotics that modulate intestinal microbiota. *Oncoimmunology.* 2016;5:e1150399.
68. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. *Orphanet J Rare Dis.* 2007;2:1.

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