

Review

Attempts to Understand Oral Mucositis in Head and Neck Cancer Patients Through Omics Studies

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Abstract: Oral mucositis (OM) is inflammation of the mouth caused by damage to the mucous membranes that line the mouth and throat. It is a side effect of cancer treatment, particularly in patients with head and neck squamous cell carcinoma (HNSCC) who undergo radiotherapy, chemotherapy, and/or immunotherapy with immune checkpoint inhibitors. The etiology and pathogenic mechanisms of OM is complex and multifaceted, involving cytotoxicity (cell death), inflammation, infection, change in microbiome, and immune-mediated cytotoxicity. We summarize the literature about attempts to use various omics methodologies (genomics, transcriptomics, microbiomics and metabolomics) to elucidate the biological pathways associated with the development or the severity of OM. Integrating different omics into multi-omics approaches carries the potential to discover links among host factors (genomics), host responses (transcriptomics, metabolomics), and local environment (microbiomics).

Keywords: Head and neck cancer; oral mucositis; pain; genomics; transcriptomics; microbiomics

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is the seventh [1] most common type of cancer worldwide. There were an estimated 54,000 new cases in the United States in 2022 [2]. The treatment of HNSCC is based largely on the primary tumor location and stage of the disease. Early-stage disease is treated with single-modality treatment, and advanced-stage disease is treated with multi-modal therapy. The majority of HNSCC patients present with loco-regionally advanced disease. The treatment modalities for HNSCC with curative intent are surgery and/or radiotherapy with or without concurrent systemic therapy. However, treatment-related toxicities are a significant concern. In 2016, the American Cancer Society guidelines [3] for HNSCC underscored the need to recognize the potential late and long-term complications or toxicities of cancer treatment, as well as its undertreatment and management.

Cancer immunotherapy using immune checkpoint inhibitors (ICIs) is a recent advance in HNSCC treatment. In recent years, there have been several combination treatments approved by the FDA for recurrent or metastatic HNSCC that include ICIs [4]: pembrolizumab in combination with platinum and fluorouracil chemotherapy, nivolumab plus ipilimumab, cetuximab plus pembrolizumab, and durvalumab plus tremelimumab. ICI monotherapy (pembrolizumab or nivolumab) is approved by the FDA for the treatment of recurrent or metastatic HNSCC that have progressed on or after platinum-based chemotherapy. Pembrolizumab is also approved as monotherapy for the first-line treatment of patients with recurrent or metastatic HNSCC whose tumors express PD-L1 and who are not candidates for platinum-containing chemotherapy. Nivolumab is also approved as monotherapy for the treatment of patients with HNSCC who have metastatic disease and are intolerant to platinum-based chemotherapy.

OM is a painful and debilitating treatment-related toxicity in HNSCC patients. OM is a common side effect of cancer treatment, particularly in patients with HNSCC who undergo radiation therapy and/or chemotherapy. The mucous membranes of the mouth and throat are highly sensitive to radiation and chemotherapy, which can damage the cells and cause inflammation. OM is clinically characterized by erythematous lesions, ulcers, and edema in the oral mucosa. This can lead to pain, swelling, difficulty eating, drinking, and speaking, and an increased risk of infection. The incidence of OM caused by chemotherapy or radiotherapy can be very high, ranging from 40% to 90% [5,6].

OM is observed to appear around a week after chemotherapy and resolve a week after the cessation of chemotherapy. Whereas among those receiving radiotherapy, a dose-response relationship is observed, with OM appearing after a cumulative dose of 30 Gy. Patients with OM complain of painful mouth ulcers and difficulty eating or swallowing. OM-related complaints lead to dose reduction or interruption of treatment, increased opioid consumption, visits to the emergency department (ED), and hospitalization. While most patients develop OM, studies show individual variability in the severity and persistence of OM despite receipt of similar cancer treatments for

HNSCC. In a recent study, up to 90% of patients developed OM, but only 36% developed severe OM[7]. Longitudinal patterns of OM also vary, with some patients experiencing early resolution[8-10] whereas others develop chronic [11] OM. Moreover, for some HNSCC patients, visits to the ED for OM-related complaints, including severe pain, occur within 7 days of the initiation of cancer treatment and may persist with pain as a chief complaint across multiple ED visits for up to 23% of HNSCC patients[12].

The above profiles of OM in HNSCC patients were based on studies prior to the common use of ICIs for HNSCC treatment. More recently, OM was also observed as treatment-related toxicity from ICI therapy[13-15]. Immune-mediated OM is caused by the immune system attacking the lining of the mouth and throat. The incidence rate of OM after ICI therapy appears to be much lower than that caused by chemotherapy or radiotherapy. The incidence rates of OM can vary depending on the specific treatment regimen, patient population, and other factors. For example, the incidence of grade 1-2 OM induced by pembrolizumab in solid tumor patients was 12.2%, and the incidence of grade 3-4 oral mucositis was 0.5%. Clinical features that may suggest immune-mediated mucositis include the timing of onset (typically within weeks to months of initiating immunotherapy), the presence of other immune-related adverse events, and the lack of response to conventional mucositis treatments such as pain relievers and topical anesthetics. Histological features that may suggest an immune-mediated cause include the presence of dense lymphocytic infiltrates and the absence of chemotherapy-related changes such as atrophy and fibrosis. If immune-mediated mucositis is suspected, a corticosteroid trial may be considered to determine if the symptoms improve with treatment. A positive response to corticosteroids would support an immune-mediated etiology and suggest that continued immunosuppressive therapy may be beneficial.

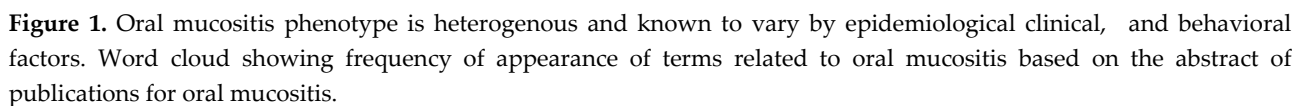
In patients with HNSCC, OM is a significant problem because it can interfere with treatment and impact their quality of life. Patients may require dose reductions or delays in their cancer treatment, which can decrease the effectiveness of the antineoplastic therapy. Additionally, OM can lead to malnutrition and dehydration, further compromising the patient's health and well-being. Therefore, effective management of OM is an important part of cancer treatment and may involve a range of supportive care measures to manage symptoms and prevent complications, such as pain management, nutritional support, oral hygiene measures, anti-infectives, and glucocorticoids. Studies continue to explore interventions[16-19] and potential treatments[20-22] for OM, showing mixed results. In 2020, the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) updated their Clinical Practice Guidelines for the management of mucositis[23]. Among HNSCC patients, the use of benzydamine (a non-steroidal drug with local anti-inflammatory, analgesic, antipyretic, and anti-edema effects) was added as a new suggestion for the prevention of OM when receiving radiotherapy and chemotherapy and a recommendation for the prevention of OM with intraoral *photobiomodulation* therapy among those receiving radiotherapy with or without concurrent chemotherapy.

Clinician-rated and patient-rated scales are used to characterize OM. Table 1 shows a list of clinician-rated and patient-rated OM scales. Self-report measures of OM severity and patient-reported outcome measures of the impact of OM on bodily function and quality of life, have also been developed and validated for use in different languages. The scales developed by the World Health Organization [24], Radiation Therapy Oncology Group [25], and US National Institutes of Health (i.e., Common Terminology Criteria for Adverse Events) [26] are the most frequently used OM scales, especially in large clinical studies. Importantly, a recent study by Villa and colleagues[27] found discrepancies in concordance between these 3 scales. However, they noted that scale selection is less critical in studies where severe mucositis is the outcome of interest, but that scale selection is particularly important if the focus is to describe the clinical trajectory of OM and its impact.

Table 1. List of clinician-rated and patient-rated OM scales.

Scale	Items
World Health Organization*[24]	0=No changes; 1= Soreness/erythema; 2= Soreness/erythema; ulceration ability to eat solid food; 3= Soreness/erythema + ulceration + ability to use a liquid diet only; 4= Soreness/erythema + ulceration + no possible oral alimentation
Radiation Therapy Oncology Group[25,28]	Grade I: Erythema; Grade II: Patchy reaction (<1.5 cm, non-contiguous); Grade III: Confluent mucositis (>1.5 cm, contiguous); Grade IV: Ulceration, necrosis, bleeding.
National Cancer Institute [26]	Grade 0 (None) None; Grade 1 (Mild) Painless ulcers, erythema, or mild soreness in the absence of lesions; Grade 2 (Moderate) Painful erythema, oedema, or ulcers but eating or swallowing possible; Grade 3 (Severe) Painful erythema, oedema, or ulcers requiring IV hydration; Grade 4 (Life-threatening) Severe ulceration or requiring parenteral or enteral nutritional support or prophylactic intubation; Grade 5 (Death) Death related to toxicity
Western Consortium for Cancer Nursing Research[29]	Lesions: none; Color: pink; Bleeding: none; 1= Lesions: 1–4; Color: slight red; Bleeding: N/A; 2= Lesions: >4; Color: moderate red; Bleeding: with eating and oral hygiene; 3= Lesions: coalescing; Color: very red; Bleeding: spontaneous;
Oral Assessment Guide[30]	Eight categories of oral health (lips, tongue, gums and tissues, saliva, natural teeth, dentures, oral cleanliness and dental pain) are assessed as healthy, changes or unhealthy.
Oral Mucositis Assessment Scale (OMAS)[31]	Graded 0–3 for ulceration and 0–2 for erythema at nine sites within the oral cavity.
The Oral Mucositis Weekly Questionnaire	8 items; Likert scale: Soreness/Pain, Activity limitation, Quality of Life
Oral mucositis daily questionnaire[32,33]	10 items; Likert scale: Overall health, Severity , Functional limitations
Oral Health Impact Profile-14 (OHIP-14)[34]	14 items; Likert scale : Functional limitation, Physical pain, Psychological discomfort, Psychological disability, Physical disability, Social disability, Handicap
MD Anderson Symptom Inventory- Head and Neck (MDASI-HN)[35]	28 items: 0–10 scale Along with the core MDASI’s 13 symptom items and 6 interference items, the MDASI-HN also assesses 9 symptoms relevant to head and neck cancer
Patient-Reported Oral Mucositis Symptoms (PROMS)[36]	10 items: Visual analogue scale : Pain, Oral functions

Epidemiological, behavioral, and clinical variables explain some of the variation observed in OM. For some patients, OM may be very painful, leading to treatment interruption, increased opioid consumption, and increased healthcare utilization. With advances in molecular technology, studies have explored biomarkers for identifying patients at risk for severe OM and assessing potential biological mechanisms of this complex trait. Although there have been studies attempting to identify markers of risk and potential therapies for OM aiming to improve quality of life, similar studies will need to be conducted to reflect the addition of immune-mediated OM into the mix of OM in HNSCC patients in this new era of cancer immunotherapy.



OM may be a classic example of gene-environment interaction, requiring an initiating event (chemotherapy, radiation, or ICI therapy) and host genetic susceptibility. As a complex human trait, it is expected that multiple genes underlie the development of OM. Assessing the interaction of these genes with epidemiological, behavioral, and clinical variables will provide a better understanding of OM and allow early identification of patients at risk. With advances in high throughput molecular laboratory techniques, it is now possible to assess biomarkers that measure events at the physiological, cellular, and molecular levels. We reviewed studies conducted from 2016–2022 to identify putative biomarkers for OM (Figure 2), for predicting the severity of OM and identifying novel therapeutic targets. The goal is to highlight the knowledge gap that is required to improve patient outcomes by enabling personalized treatment strategies and reducing the burden of OM.

2. "Omics" approaches may help in risk assessment and developing personalized and more effective therapies OM

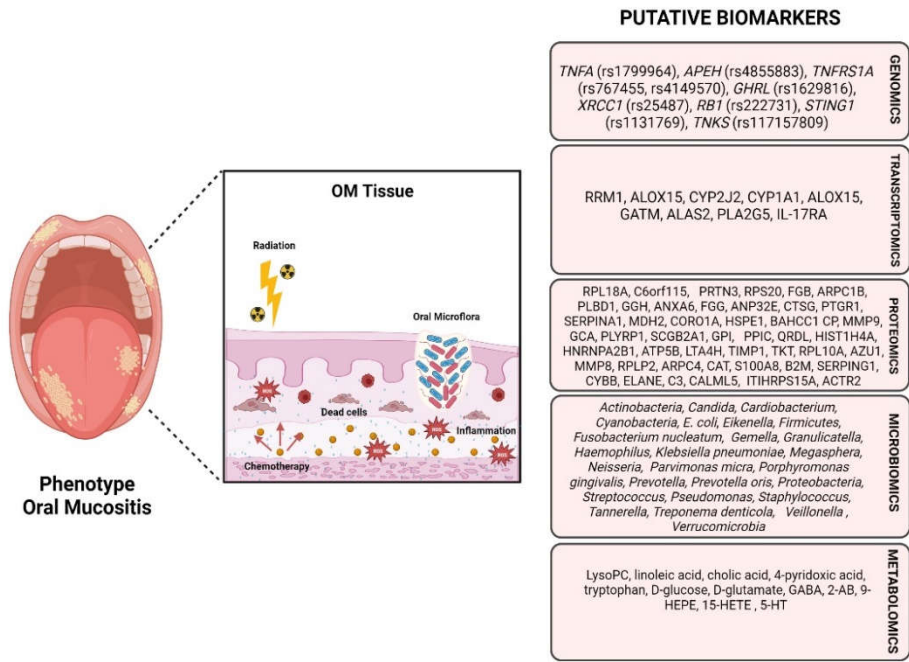


Figure 2. Putative biomarkers identified using the different omics approaches.

2.1. Genomics

The Human Genome Project and the HapMap Project gave a rough sketch of how people's genes differ [37]. The Telomere-to-Telomere Consortium completed sequencing the last 8% of DNA of the human genome, i.e., the part of the human genome not covered by the Human Genome Project, in 2022 [38]. Now the complete blueprint of the human body can accelerate the advance in the study of human biology and medicine. Single nucleotide polymorphisms (SNPs), which happen about once every 1000 bases in a genome with 3 billion base pairs, are the most common type of DNA variation. SNPs that change how the gene product works are usually further examined because they may make individuals more likely to get sick or change how they react to drugs. Also, SNPs with a minor allele frequency of more than 5% are chosen because they have a higher chance of being linked to complex diseases. Both hypothesis-driven (candidate-gene, pathway-based) and hypothesis-generating (i.e., genome-wide association) approaches have been used to identify genes for OM.

2.1.1. Hypothesis-driven Approach

In a hypothesis-driven approach, researchers use knowledge of polymorphisms and gene functions in the candidate gene, investigating one or a few selected polymorphisms at a time.

2.1.2. Candidate-Gene Studies

Studies have demonstrated associations between gene polymorphism, DNA repair function, and sensitivity to radiotherapy. Using the search terms "SNPs and mucositis" and "gene(s) and mucositis", Reyes-Gibby et al. [39] conducted a literature search of human studies of OM published before 2016. Their review of the literature identified 27 genes across the various studies, with the most commonly cited genes involved in methylation, DNA synthesis, and DNA repair mechanisms, i.e., X-ray repair cross-complementing gene 1 (*XRCC1*), and excision repair cross complementation group 1 (*ERCC1*).

A subsequent review of the literature for the period 2015–2022 showed studies focusing on HNSCC patients who received radiation therapy. Five studies were conducted on a small number of HNSCC patients receiving radiation therapy in the oncology department at the Medical University of Lublin (Lublin, Poland). They showed that

polymorphisms in *GHRL* (rs1629816) [40], *TNFRS1A* [41] (rs4149570, rs767455) [42]), *TNFA* (rs1799964) [43], and *APEH* (rs4855883) [44] were significantly associated with OM. In a larger sample (n = 114) of Chinese patients with HNSCC receiving radiotherapy, Chen et al. [45] found the *XRCC1* variant (rs25487) to be associated with an increased risk of OM.

2.1.3. Pathway-Based

Although comprehensive, the pathway-based approach relies on a priori knowledge of SNPs and gene functions and biological plausibility. Reyes-Gibby et al. [39] used a pathway-based approach for OM. They first reviewed literature before 2015 on genetic association studies of OM. They found 27 genes from 28 published studies. Using the 27 genes, they generated gene networks for OM using Ingenuity Pathway Analysis (IPA), which is a bioinformatics tool using the Ingenuity Knowledge Base comprised of information on biomolecules and their relationships. They found *TP53*, *CTNNB1*, *MYC*, *RB1*, *P38 MAPK*, and *EP300* as the most biologically significant molecules and the uracil degradation II (reductive) and thymine degradation pathways as the most significant biological pathways. They then conducted a genetic association study for OM in 885 HNSCC patients (with OM = 186; without OM = 699) utilizing 66 SNPs within the 8 most connected IPA-derived candidate molecules. The top-ranked gene identified through this association analysis was RNA-binding proteins (RBP) (rs2227311, p-value = 0.034, odds ratio = 0.67). To date, there has been a limited application of the pathway-based approach in the study of OM.

Table 2. Summary of identified genes and corresponding SNPs that were found to be implicated in oral mucositis in head and neck cancer patients.

Year	First Author	Sample Size	Sample	Phenotype	Genes	SNPs
2022	Schack[46]	Discovery=1183 Danish Cohort Replication=597 Danish Cohort Validation=235 Asian Cohort	Buffy coats	Mucositis 0: no 1: erythema 2: patchy 3: confluent 4:ulceration	STING1	rs1131769
2020	Mlak[42]	60	Peripheral blood	Mucositis RTOG/EORTC	TNFRS1 A	rs767455
2020	Mlak[43]	62	Peripheral blood	Mucositis RTOG/EORTC	TNF alpha	rs1799964
2020	Yang[47]	960 560	Blood	RTOG/EORTC	TNKS	rs117157809
2018	Brzozowska[44]	62	Peripheral blood	Mucositis RTOG/EORTC	APEH	rs4855883
2018	Brzozowska[41]	58	Peripheral blood	Mucositis RTOG/EORTC	TNFRS1 A	rs4149570
2018	Brzozowska[40]	65	Peripheral blood	Mucositis RTOG/EORTC	GHRL	Rs1629816
2017	Chen[45]	114	Peripheral blood	Mucositis RTOG/VRS	XRCC1	rs25487
2017	Reyes-Gibby[39]	885	Peripheral blood	Oral Mucositis (ICD)	RB1	rs2227311

RTOG-Radiation Therapy Oncology Group; WHO-World Health Organization; EORTC-European Organization for Research and Treatment of Cancer; ICD-International Classification of Diseases.

2.1.4. Genome-wide Approach

Hypothesis-free, whole genome-wide association studies are effective for identifying genetic factors contributing to complex diseases. However, there has been little use of this approach in research because of the cost and the need for very large sample sizes for replicable results. A study by Schack et al.[46] utilized 3 cohorts (discovery phase: 1183 Danes, replication phase: 597 Danes, and validation phase: 235 Asians) and found the rs1131769 of the STimulator of Interferon Response cGAMP Interactor 1 gene (*STING1*) to be significantly associated with OM. *STING1* (also known as transmembrane protein 173) has been associated with infection, inflammation, immunity, autophagy, and cell death

[48-51]. An earlier genome-wide study by Wang et al. [47] found SNP rs117157809 in the protein-coding gene TNKS (Tankyrase) associated with more than three-fold OM risk in patients with nasopharyngeal cancer. TNKS plays a role in radiation-induced damage. Depletion of TNKS is associated with increased sensitivity to ionizing radiation-induced mutagenesis, chromosome aberration, telomere fusion and cell-killing [52].

2.2. Microbiomics of OM

The application of microbiomics has been used in OM research in HNSCC patients since the microbiome (the collective community of microorganisms) in the oral cavity is implicated in the development of OM. It is hypothesized that changes in the composition of the oral microbiome can increase inflammation and tissue damage in the oral mucosa.

Reyes-Gibby et al. [7] identified different features associated with the risk of OM at baseline (*Cardiobacterium*, *Granulicatella*), immediately before the development of OM (*Prevotella*, *Fusobacterium*, *Streptococcus*), and immediately before the development of severe OM (*Megasphaera*, *Cardiobacterium*). Interestingly, *Prevotella* and *Fusobacterium* have also been identified in the study of Hou et al. [53], where these features showed dynamic synchronous variations in abundance throughout the course of radiation therapy and frequently coincided with the onset of severe OM.

The combination of microbiomic and genomic data may also be a powerful approach to identifying key features for therapy. For example, in 24 HNSCC patients who received radiotherapy and concomitant chemoradiotherapy, microbial species (*Staphylococcus aureus*, *S. epidermis*, *Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae*) from saliva samples expressed higher levels of antibiotic-resistance genes (VIM2, MCR-1, TET(K), blaKPC) after receiving cancer therapy [54].

Microbiomics has emerged as an important field of research in the study of OM, despite several challenges. For example, the interpretation of microbiome data can be challenging with the utilization of complex tools, a lack of standardization, and highly variable data that are easily influenced by factors such as diet, medications, and even oral hygiene. Moreover, observed changes in the oral microbiome associated with OM are not adequate to establish their causal or modifier roles in OM. Treatment-induced dental caries, hyposalivation, and xerostomia may change the environment in the upper orodigestive tract to affect the composition of the microbiome.

2.3. Metabolomics

Metabolomics involves the comprehensive characterization of low-molecular-weight molecules, metabolites, and metabolism in biological systems. Studies have collected saliva, serum, and volatile organic compounds to reflect various pathological conditions, making it an attractive source for the diagnosis of systemic diseases and potential biomarkers of pathological conditions. Analytical platforms such as liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), capillary electrophoresis time-of-flight mass spectrometry (CE-TOF-MS), and nuclear magnetic resonance (NMR) spectroscopy are commonly used to measure and profile small molecule metabolites [55]. In OM research, metabolomics provides valuable insights into the biochemical changes that occur in the oral mucosa as a result of radiation or chemotherapy treatment.

Yatsuoka et al. [56] (2021) analyzed the time course of salivary metabolic profiles using CE-TOF-MS in nine male patients with HNSCC who received radiation therapy of at least 50 Gy. Partial least squares regression-discriminant analyses showed that histidine and tyrosine highly discriminated between high-grade OM and low-grade OM at baseline. Moreover, γ -aminobutyric acid (GABA) and 2-aminobutyric acid (2AB) concentrations were higher in the high-grade OM group than in the low-grade OM group. While GABA is known to be correlated with stress levels, 2AB is known to increase under high oxidative stress conditions, which can be induced by radiation.

The metabolomic changes brought about by maxillofacial and oral massage (MOM) attempting to attenuate severe radiotherapy-induced OM in patients with nasopharyngeal carcinoma were also explored by Yang et al. [57]. They identified enhanced levels of the metabolites 9S-HEPE and 15-HETE among patients receiving MOM after radiotherapy, both of which are known to play a role in inhibiting inflammatory responses through different pathways [58].

Animal OM models have also been useful in understanding the pre-clinical potential of pharmacological targets. Two independent studies involving the use of Chinese herbal medicine, Shuanghua baihe tablet (SBT) and Kouyanqing granules (KQG), have used OM rat models to determine the effects in alleviating OM symptoms and elucidate the potential metabolic pathways involved. Geng et al. (2021) [59] showed the role of SBT in metabolic-related pathways such as linoleic acid and cholic acid metabolism to alleviate the inflammatory symptoms of OM. KQG, on the other

hand, shows promising effects by attenuating symptoms of oral ulcers through regulation of the neuroimmunoendocrine system oxidative stress, and tryptophan metabolism [60].

2.4. Proteomics and transcriptomics

Transcriptome analysis typically investigates differential gene expressions that occur in different (e.g., normal vs. abnormal) states. Transcriptomic tools make use of high-throughput technologies such as microarrays, RNA sequencing, and, more recently, single-cell transcriptomics, wherein an individual cell is profiled.

Table 3. Summary of omics approaches and identified putative biomarkers for therapy-induced oral mucositis in head and neck cancer.

Year	First Author	Phenotype	Samples	Sample size	Methods	Targets	Results
Metabolomics.							
<i>Animal Models</i>							
2021	Geng [59]	Mucositis (0-5)	Serum from OM rat model, induced with 5-FU and 10% acetic acid	30 rats	UHPLC	Cholic acid, linoleic acid, 4-pyridoxic acid, LysoPC	Shuanghua Baihe tablets improve inflammatory symptoms of oral mucositis.
2020	Chen	Induced oral ulcers and degree of healing	Serum from OM rat model, induced with 15% chloral hydrate	42 rats	LC-QTOF/MS	5-HT, GABA	Kouyanqing granules attenuate the symptoms of oral ulcers worsened by sleep deprivation through regulation of the neuroimmunoendocrine system, oxidative stress levels, and tryptophan metabolism.
<i>Clinical Samples</i>							
2021	Yatsuoka [56]	NCI CTCAE	Saliva	9 HNC	CE-TOF-MS	tryptophan, D-glucose, D-glutamate, GABA, 2-AB	Pre-treatment concentrations of gamma-aminobutyric acid and 2-aminobutyric acids were higher in the high-grade OM group.
2021	Yang [57]	NRS 0-10	Peripheral blood	10 NPC	UHPLC-MS/MS	9-HEPE, 15-HETE	MOM promotes the release of anti-inflammatory lipids to reduce tissue damage; enhancement of 9S-HEPE and 15-HETE in all radiation doses.
Microbiomics							
2020	Reyes-Gibby [7]	NCI CTCAE	buccal mucosal	66 Locoregional HNSCC	16S rRNA	<i>Cardiobacterium</i> , <i>Granulicatella</i> , <i>Prevotella</i> , <i>Fusobacterium</i> , <i>Streptococcus</i> , <i>Megasphaera</i> , <i>Cardiobacterium</i>	Genera abundance was associated with the hazard for the onset of severe OM.
2020	Vesty [61]	WHO	saliva and oral swabs	19 HNC	NGS	<i>Fusobacterium</i> , <i>Haemophilus</i> , <i>Tannerella</i> , <i>Porphyromonas</i> and <i>Eikenella</i> , <i>Candida</i>	Gram-negative bacteria on the buccal mucosa may influence susceptibility to developing OM.
2019	Subramaniam and Muthukrishnan [54]	WHO	unstimulated whole saliva	24 HNSCC	16S rRNA	<i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i>	The bacterial isolates obtained during and at the end of therapy appeared to express a higher level of antibiotic-resistance genes (<i>VIM2</i> , <i>MCR-1</i> , <i>TET[K]</i> , <i>blaKPC</i>) than those isolated at the onset of therapy.
2018	Hou [53]	RTOG	oral swabs	19 NPC	16S rRNA	<i>Prevotella</i> , <i>Fusobacterium</i> , <i>Treponema</i> , <i>Porphyromonas</i>	<i>Prevotella</i> , <i>Fusobacterium</i> , <i>Treponema</i> and <i>Porphyromonas</i> showed dynamic synchronous variations in abundance

Year	First Author	Phenotype	Samples	Sample size	Methods	Targets	Results
							throughout the course of radiation therapy, frequently coinciding with the onset of severe mucositis.
2017	Zhu [62]	RTOG	oral or retropharyngeal mucosa swabs	41 NPC	16S rRNA	<i>Firmicutes, Proteobacteria, Bacteroidetes, Fusobacteria, Actinobacteria, Spirochaetes, Cyanobacteria, Verrucomicrobia, Acidobacteria, TM7, Deinococcus-Thermus</i> and SR1	Oral microbiota changes correlate with the progression and aggravation of radiotherapy-induced mucositis in patients with nasopharyngeal carcinoma.
Microbiota							
2018	Almstahl [63]	WHO	Swab culture	33 HNC	Culture	<i>Neisseria, Fusobacterium, Prevotella, Candida</i>	Levels of <i>Neisseria</i> decreased and mucosal pathogens increased during RT; 2 years post-treatment, <i>Fusobacterium</i> and <i>Prevotella</i> decreased; growth of <i>Candida</i> increased
2018	Gaetti-Jardim [64]	NCI CTCAE	Supra and subgingival biofilms	28 HNC	Culture	<i>Candida, Enterobacteriaceae</i>	<i>Candida</i> and family <i>Enterobacteriaceae</i> showed increased prevalence with RT, and were associated with the occurrence of mucositis and xerostomia
Transcriptomics							
Animal Models							
2021	Geng [59]	Mucositis (0-5)	Serum from OM rat model, induced with 5-FU and 10% acetic acid	30 rats	Whole genome sequencing	ALOX15, CYP2J2, CYP1A1, ALOX15, GATM, ALAS2, PLA2G5	Shuanghua Baihe tablets improve inflammatory symptoms of oral mucositis.
2021	Saul-McBeth [65]	Induced oral ulcers, % damage	OM mice model; Induced with head and neck irradiation	3 mice	RNA Seq	IL-17RA	IL-17RA provides protection during HNI-induced OM by preventing excess inflammation during ulceration phase of OM.
Clinical Samples							
2018	Mlak [66]	RTOG/EORTC	Plasma	60 HNC	Microarray	RRM1	RRM1 gene expression in cfRNA allows for estimating risk of severe OM.
Proteomics							
2015	Jehmlich [67]	NCI CTC v3	Unstimulated whole saliva	50 HNC	MS	RPL18A, C6orf115, PRTN3, RPS20, FGB, ARPC1B, PLBD1, GGH, ANXA6, FGG, ANP32E, CTSG, PTGR1, SERPINA1, MDH2, CORO1A, HSPE1, BAHCC1 CP,	48 proteins differed significantly between OM group and non-OM group. 17 proteins displayed increased levels and 31 proteins decreased in level in OM.

Year	First Author	Phenotype	Samples	Sample size	Methods	Targets	Results
						MMP9, GCA, PLYRP1, SCGB2A1, GPI, PPIC, QRDL, HIST1H4A, HNRNPA2B1, ATP5B, LTA4H, TIMP1, TKT, RPL10A, AZU1, MMP8, RPLP2, ARPC4, CAT, S100A8, B2M, SERPING1, CYBB, ELANE, C3, CALML5, ITIHRPS15A, ACTR2	

NCI CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events, NRS: numerical rating scale; WHO: World Health Organization; RTOG: Radiation Therapy Oncology Group; EORTC: European Organization for Research and Treatment of Cancer; 5-FU: 5-fluoruracil; HNC: head and neck cancer; NPC: nasopharyngeal cancer; HNSCC: head and neck squamous cell carcinoma; UHPLC: ultra-high-performance liquid chromatography; LC-QTOF/MS: liquid chromatography- quadrupole time-of-flight (TOF)/mass spectrometer (MS); CE-TOF-MS: capillary electrophoresis-TOF-MS; NGS: next generation sequencing; 5-hydroxytryptamine; GABA: γ -aminobutyric acid; 2-AB: 2-aminobutyric acid; 9-HEPE: 9-hydroxyeicosapentanoic acid; 15-HETE: 15-hydroxyeicosatetraenoic acid; RT: radiotherapy; OM: Oral mucositis; MOM: maxillofacial and oral massage.

Transcriptomic data aid in the elucidation of the mechanisms involved in OM. By analyzing patterns of gene expression, dysregulated key pathways and biomarkers can be identified. For example, microRNAs (miRNA) are small non-coding RNAs that play an important role in post-transcriptional gene regulation by binding to specific mRNA molecules and initiating degradation or translational inhibition, thereby affecting gene expression patterns. In the context of OM, the miR-1206 variant has been associated with a 3-fold increase in the risk of developing methotrexate-induced OM [68], whereas miR-200c showed promising results in reducing ROS production and repressing proinflammatory cytokines in animal models [69].

While transcriptomics and proteomics provide different types of data, there is a significant overlap between the two approaches, since changes in gene expression may lead to changes in protein expression, and changes in protein expression can also regulate other downstream gene expression and protein levels. By integrating data from both approaches, we can gain a more complete understanding of the biological processes, networks, and pathways involved in OM and identify targets for the treatment or prevention of therapy-induced OM.

The proteomic approach for OM research has focused on biomarker development, particularly on inflammatory proteins observed after treatment-induced OM. Kiyomi et al.[70] utilized a bead array and an enzyme-linked immunosorbent assay (ELISA) to identify potential biomarkers of OM from saliva or oral swab samples were taken from 20 leukemia or head and neck cancer patients undergoing treatment. Their study suggests that salivary IL-6, IL-10, and TNF- α may serve as predictors of OM occurrence and grade. Additionally, Jehmlich et al. [71] were able to identify 48 (see Table 3) unique proteins that differ significantly between OM and non-OM groups with saliva and/or oral swabs obtained from a pool of 50 head and neck cancer patients. Among those identified is proteinase 3 (PRTN 3), a secretory multifunctional serine protease that can degrade elastin, fibronectin, and collagen. PRTN3 is released upon neutrophil activation and degranulation during tissue injury inflammation. This has also been implicated in the serum proteomic profile of HNSCC patients. While different protein profiles were obtained from the patients due to variations in tumor status and collection time points, most of the 48 proteins are extracellular and have important roles in inflammatory and innate immune responses, and complement activation cascade [71].

3. Conclusions

These different types of “omics” studies provide preliminary evidence that high-throughput methodologies applied to study different aspects of the host (host biomarkers), response to cancer treatment (chemotherapy and/or radiotherapy), and microbiological factors (microbiome and infection) are feasible. There is a paucity of these studies, and there is a complete lack of studies that integrate and analyze multi-omics data to examine the importance of genomic variations, gene-environment interactions, and mechanisms of the host response to a chemical or radiational cytotoxicity to the development of OM. Moreover, the addition of ICI therapy to the mix of antineoplastic treatment for HNSCC adds immune-mediated etiology into the pathogenic mechanism of OM, and this mechanism has not been studied in HNSCC patients.

4. Future Directions

The basic principle of molecular epidemiology [72] is that neither genetics nor environment alone is responsible for individual variation in disease presentation and severity. Whereas traditional field-based epidemiological approaches have identified subgroups at higher risk for OM (older age, body mass index, radiotherapy dose), the development of high throughput molecular laboratory techniques has allowed for the use of biological markers in disease prevention and risk prediction. These biomarkers measure events at the physiological, cellular, and molecular levels, thus improving our understanding of the epidemiology of diseases. Integrating the use of molecular epidemiology methods allows for a better understanding of biological mechanisms and improves assessments of individual risks by providing person-specific information (a genetic profile, etc.) along with clinical

information. Such tools could identify subgroups who might benefit most from intervention and contribute to developing personalized and more effective therapies while reducing toxic side effects.

Applying omics approaches may potentially identify subgroups of patients who will benefit from a specific intervention or treatment. However, a common limitation of the studies was the heterogeneity of the population sample, the small sample size, the use of different OM measures, and the retrospective study design.

Challenges include the heterogeneity of the underlying pathogenic mechanisms of OM given that there are now three main types of cancer treatment for HNSCC (chemotherapy, radiotherapy, and ICIs) that can cause OM. Prospective studies that will allow assessment of the timing of OM onset and the response to conventional mucositis treatments are needed to identify pathogenic mechanisms. In addition, the clinical features of OM can help identify the involvement of infection (viral, fungal, or bacterial) or immune reaction, ie. lesion appearance, location, redness, swelling, ulceration, pain severity, etc. The use of statistical approaches such as hierarchical clustering to assess which clinical features of OM will correlate with different multi-omics patterns will provide insight into the pathogenic mechanisms and potential treatment targets.

Importantly, omic studies are resource intensive and therefore, it is important to note that funding announcements by the US National Institute of Dental and Craniofacial Research (NIDCR) and the US National Cancer Institute (NCI) may provide the opportunities to pursue multi-center studies applying omics approaches to OM. In particular, a reissue by NIDCR of [PAR-17-154](#)[73] calls for prospective observational designs and biomarker validation studies. Considered appropriate would be epidemiologic studies of disease prevalence or incidence, cohort studies prospectively ascertaining risk factors for disease development, cohort studies that provide longitudinal follow-up of treatment outcomes, case-control studies with longitudinal follow-up, and large cross-sectional studies or case-control studies evaluating genomic changes, gene-environment interactions, or disease/treatment mechanisms through -omics, cellular, and imaging analyses. For the biomarker validation studies, the reissue of [PAR-17-154](#) will[73] promote advanced analytic and/or clinical validation of strong candidate biomarkers and endpoints for the diagnostic or prognostic utility to demonstrate that biomarker or endpoint change is reliably correlated with pathophysiology, clinical outcome, therapeutic target engagement or treatment response.

Author Contributions: Conceptualization, C.C.R.G.; writing—original draft preparation, C.C.R.G., E.M.D.S.V., and S.J.Y.; writing—review and editing, C.C.R.G., E.M.D.S.V., and S.J.Y.; visualization, E.M.D.S.V.; funding acquisition, C.C.R.G., S.J.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was partially supported by grants from the National Institutes of Health (NIH) to Drs. Reyes-Gibby (R01CA267856; R21DE026837) and Yeung (R01CA267856).

Data Availability Statement: Data sharing not applicable.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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