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Salivary biomarkers for detection of oral squamous cell carcinoma – current state and recent advances

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Abstract

Oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of the oral cavity. Detection of OSCC is currently based on thorough clinical oral examination combined with biopsy for histological analysis. Most cases of OSCC are not detected until the cancer has developed into advanced stages; thus, a reliable early stage diagnostic marker is needed. This literature review presents an overview of the status of current advances in salivary diagnostics for OSCC. Though many protein and mRNA salivary biomarkers have been identified that can detect OSCC with high sensitivity and specificity, the most discernable findings occur with the use of multiple markers. Studies that incorporate proteomic, transcriptomic, and potentially additional "omics", including methylomics, need to be initiated to bring technology to clinical applications and allow the best use of saliva in diagnosing OSCC.

Keywords

salivary diagnostics; salivary biomarker; oral fluid diagnostics; oral squamous cell carcinoma; oral cancer; salivaomics; transcriptomics; proteomics; microbiomics; methylomics; metabolomics; exosomes

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Conflict of Interest

Dr. Maha Yakob received a grant from NIH.
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Dr. David T.W. Wong is co-founder of RNAmeTRIX Inc., a molecular diagnostic company. He holds equity in RNAmeTRIX, and serves as a company Director and Scientific Advisor. The University of California also holds equity in RNAmeTRIX. Intellectual property that David Wong invented and which was patented by the University of California has been licensed to RNAmeTRIX. Additionally, he is a paid consultant to PeriRx.

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

Oral cancers account for 2–4% of all cancer cases worldwide, with approximately 40,000 new cases and 8,000 deaths in the United States in 2013 [1–5]. Oral squamous cell carcinomas (OSCC) represent the most frequent of all oral neoplasms, and more than 90% of all oral neoplasms are estimated to be OSCC [6,7]. The 5-year survival rate of oral cancer is 60–80% when detected during its early stages [1, 7].

OSCC is acquired from a combination of environmental risk factors and genetic predispositions. In combination with an individual's genetics, exposure to tobacco, alcohol, and radiation, among other carcinogens, has the ability to mutate oncogenes that are in charge of cell survival and proliferation [8–10]. Clinically defined lesions of the oral mucosa have a higher oncogenic risk than normal oral mucosa. Lesions termed leukoplakia, erythroplakia, and leukoerythroplakia are potentially malignant lesions of the aerodigestive tract [11]. These lesions are defined as dysplasia of variable grades when verified with cellular atypia but without invasion. However, the values that predict cancer occurrence are a matter of debate because OSCC can arise where any epithelial dysplasia is detected [12].

Surgery and radiotherapy are currently the primary treatments. Surgical therapy has significant effects on swallowing, speech, and physical appearance, greatly affecting the patient's quality of life. Patients who have undergone treatment for OSCC are followed up regularly to detect recurrence. Recurrence occurs in 15–33% of patients [13,14], with local recurrence being more common; thus, an improved diagnostic tool to predict which patients are most at risk for OSCC recurrence is needed [13,15]

Detection of OSCC is currently based on thorough clinical oral examination combined with biopsy for a histological exam if an abnormal area is detected. The location from which the biopsy sample is taken is crucial for histopathological verification of the oral cancer. However, selecting the right location is difficult because of the non-uniform appearance of cancerous and precancerous lesions.

DNA mutations have also been observed in epithelial cells with no evidence of histopathological changes, showing that the current tools for detecting altered epithelial cells, such as clinical exam and histopathology, are not enough for predicting areas at high risk of developing oral cancer [16–18].

Early detection and diagnosis lead to a greater survival rate and play a significant role in successful clinical treatment. Most OSCC cases are detected when the cancer has developed into the advanced stages. Some lesions are difficult to detect in a general examination if located in hard to find regions. Delayed detection may account for the high morbidity rate of OSCC [7,19,20]. OSCC is particularly hard to diagnose early because the early stages may be painless and a burning sensation may not develop until the neoplasm has advanced [2]. This literature review will present an overview of the advances and most recently published papers on salivary diagnostics for OSCC.

Saliva composition and method of collection

Whole saliva (WS) is a mixture of fluids produced and secreted by major and minor salivary glands in the mouth and throat. WS contains proteins, microorganisms, cellular debris, gingival crevicular fluid, and serum components [21]. The paired major glands include the parotid, submandibular, and sublingual glands and account for the majority of saliva volume [22]. However, the minor glands, which produce less than 10% of the total saliva volume, comprise most of the mucosal protective component of saliva. The mucosal salivary glands are responsible for making mucins, a class of salivary glycoproteins that layer and lubricate dental surfaces to protect the mucosa from mechanical wear, and they have antiviral, antifungal, and antibacterial implications [23]. The main component of saliva is water (99.5%), with proteins (0.3%) and inorganic species (0.2%) making up a small portion of WS. Salivary analysis is inherently challenging because not only are the potential biomarkers available in small amounts, but the concentrations of different markers vary from milligrams to picograms per milliliter [21,24].

Saliva collection is non-invasive, simple, and rapid. Saliva can be collected in two ways: “unstimulated” or “stimulated”. Unstimulated WS is collected by draining or drool, spitting, suction, or swab. Stimulated saliva is collected by providing the patients with a stimulant agent, such as citric acid, paraffin, or a gum base. With stimulated collection, saliva is obtained primarily from the parotid gland, whereas unstimulated (resting) saliva is produced primarily by the submandibular gland, with minor contributions from the parotid and sublingual glands [25,26]. In addition, stimulation of saliva production decreases the concentration of small molecules, such as myoglobin, changing the total composition of the analyzed saliva in favor of larger molecules [27]. Thus, unstimulated saliva is more favorable for biomarker discovery and has been utilized in most diagnostic studies [28].

Translational applications of salivary diagnostics

Saliva is a multi-constituent oral fluid capable of mirroring both oral and systemic health conditions. Salivary analysis has been shown to be a useful diagnostic tool for other distant malignancies, including breast cancer [29], lung cancer [30–32], Sjögren’s syndrome [33], and pancreatic cancer [34,35].

Saliva contains biomarkers, which can be used as indicators of disease. According to the National Institutes of Health (NIH), a biomarker is a characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process, or pharmaceutical response to therapeutic intervention. A biomarker must be verified and validated before it can be used in a clinical assay and have any impact or application in health risk assessment [36,37].

In biomarker research, the sensitivity and specificity of a marker must be determined in each study. Sensitivity is the true-positive rate, which is described by the percentage of the total number of people with the disease that test positive. Specificity is the true-negative rate, which measures the proportion of individuals that test negative for the disease that actually do not have the disease. The area under the receiver operating characteristics (ROC) curve (AUC) is also an important measurement when reporting biomarker performance. The AUC

for a biomarker diagnostic test can range from 50%, which correlates to having no better insight than chance alone, to 100%, which denotes a perfect diagnostic test [38,39].

Current state of OSSC-specific salivary biomarkers

Given that saliva is in direct contact with the oral mucosa and cancerous lesions, the screening and detection of early OSCC lesions using saliva has promise. Salivary diagnostics may avoid many unnecessary biopsies, as well as hospital and outpatient clinical visits. Existing therapy for OSCC patients is based on traditional stage-predicting guides (mostly the TNM criteria) and histological grading [40–43]. An important advancement in salivary diagnostics is the development of omics-based biomarkers. The term salivaomics was coined to reflect the rapid development of translational and clinical tools based on salivary biomarkers [44*].

PROTEOMICS

Recent developments in proteomic technologies, such as mass spectrometry, liquid chromatography, and protein/peptide labeling technologies, allow the detection of low abundance molecules in the saliva proteome [45,46]. Numerous studies have reported that the proteomic profile of saliva from OSCC patients differs from the profile for OSCC-free controls (Table 1).

In 2008, 1166 salivary proteins were initially identified in a National Institute of Dental and Craniofacial Research (NIDCR)-funded project that sought to catalog and annotate the human salivary proteome [47]. This project was an essential first step for saliva to be clinically useful in disease diagnosis and health monitoring. The majority of the proteins are synthesized and subsequently secreted into the oral cavity by the salivary gland acinar cells [48]. This observation suggests that proteomic constituents of saliva are products of the salivary glands, which may be subject to internal and external factors. Consequently, the salivary proteome has been useful for identifying biomarkers for both local and distant diseases [49].

The matrix metalloproteinases (MMP) may play a key role in cancer development, as they cause degradation of the extracellular matrix and basement membranes. MMPs have been studied as potential cancer biomarkers and been associated with tumor invasion and metastasis [50]. In a recent study, Stott-Miller et al. determined whether salivary concentrations of the most highly differentially expressed MMPs could be used as a diagnostic aid. The concentrations of MMP1 and MMP3 were tested in saliva samples from 100 subjects (60 primary OSCC cases, 15 dysplasia cases, and 25 controls). The protein concentrations were higher in the saliva from OSCC patients compared to the saliva from cancer-free controls [51]. In another study, MMP-9 levels were elevated in 19 tongue cancer patients, as well as the levels of carbonyls, mammary serine protease inhibitor (Maspin), and Cyclin D1 (Cyc D1) [52]. The tongue is the most common site of OSCC, accounting for almost half of all OSCC cases [42, 53].

Cytokines are intercellular signaling proteins that play a role in normal growth, cellular proliferation, tissue repair, and angiogenesis. Cytokines are also involved in the immune

response against infection and inflammation. Rheumatoid arthritis, osteoporosis, diabetes, and periodontal disease can increase inflammatory protein levels. However, Interleukin-8 (IL-8) levels have been reported to be significantly higher in saliva from OSCC patients compared to patients with severe periodontal disease. Both IL-1β and IL-8 were reported to be significantly higher in OSCC patients compared to matched healthy control subjects. The research group used Luminex xMAP, which was shown to be as effective as enzyme-linked immunosorbent assay (ELISA) for the quantification of saliva proteins. Luminex xMAP technology has the advantage of high sensitivity, throughput, efficiency, and is less time-consuming than ELISA [46]. In a study focusing on tongue squamous cell carcinoma (TSCC) and salivary biomarkers, five cytokines (IL-1α, IL-6, IL-8, VEGF-A, and TNF-α) were elevated in patients with TSCC compared to controls [54].

In 2008, a panel of candidate protein biomarkers for the detection of OSCC was identified by immunoassay validation. The combination of five candidate protein markers, myeloid-related protein 14 (MRP14), profiling, CD59, catalase, and Mac-2-binding protein (M2BP), had a sensitivity of 90% and specificity of 83% for OSCC detection, showing that the proteomic profile of saliva from OSCC patients differs from that of OSCC-free controls [55]. Mass spectrometry-based proteomics was used to discover differences in salivary protein abundance between subjects with pre-malignant and malignant oral lesions. Biochemical validation showed that myosin and actin are promising salivary biomarkers capable of discriminating malignant oral lesions. Actin and myosin are key cytoskeletal proteins that facilitate cell motility and invasion, behavior central to epithelial tumorigenesis [56].

In another study, salivary biomarkers for early stage OSCC were identified by two-dimensional gel electrophoresis and mass spectrometry, and then validated by Western blot analysis and ELISA. Transferrin levels were elevated in the saliva from a mostly male sample of 41 OSCC patients compared to 30 OSCC-free controls. The increase in salivary transferrin correlated with increasing tumor size. Transferrin is needed for the growth of rapidly growing cells and is involved in DNA synthesis, electron transport, mitogenic signaling pathways, proliferation, and cell survival [57].

Fibroblast growth factors (FGFs) are heparin-binding proteins involved in angiogenesis, wound healing, embryonic development, and various endocrine signaling pathways. FGFs are key players in the proliferation and differentiation of a wide variety of cells and tissues. Basic fibroblast growth factor (bFGF) is a solid mitogen that stimulates the proliferation of cells of mesodermal and neuroectodermal origin and is reported to be involved in wound healing, hematopoiesis, angiogenesis, and tumor progression [58–60]. Salivary bFGF levels were found to be significantly elevated in patients newly diagnosed with OSCC compared to healthy controls. The findings suggest that bFGF could be a potential biomarker for the detection of OSCC in patients with no oral mucosal disease, such as oral lichen planus (OLP), geographic tongue, aphthous ulcer, or candidiasis. Salivary bFGF could also be used to detect recurrence, as the levels have been reported to be higher in patients with newly diagnosed OSCC than in those who had completed treatment and not exhibited any recurrence for at least 2 years [61].

Another recent study compared the ratio of total sialic acid to total protein (TSA/TP) and alpha-L-fucosidase activity in saliva from patients with cancer in the oral cavity, those with oral precancerous conditions, and healthy controls. The salivary TSA/TP and alpha-L-fucosidase activity were significantly increased in patients with precancerous conditions and cancer compared to the controls ($p=0.005$ and $p=0.001$, respectively) [62].

TRANSCRIPTOMICS

Evaluation of the salivary transcriptome and extracellular RNA is an emerging diagnostic technology due to its discriminatory power for disease detection. High-throughput microarray technology has made the investigation of gene expression on a genome-wide level feasible and routine. In 2004, microarray analysis showed that more than 1600 genes are significantly differentially expressed in saliva from OSCC patients and controls [63]. This pioneering study provided salivary transcriptome diagnostics with new opportunities for the early diagnosis of oral cancer and other human diseases. That study reported potential salivary RNA biomarkers, and the combinations of these biomarkers yielded a sensitivity of 91% and specificity of 91% for distinguishing OSCC from controls [63]. Recently, these biomarkers were studied again in a Serbian population and five independent cohort populations in Los Angeles [64**,65**]. The RNA biomarkers included *IL-8*, *IL-1β*, Dual specificity phosphatase 1 *(DUSP1)*, Ornithine decarboxylase antizyme 1 *(OAZ1)*, Spermidine/spermine N1-acetyltransferase 1 *(SAT1)*, S100 calcium binding protein P *(S100P)*. The sensitivity and specificity of the combination of biomarkers remained high in both cases, further enforcing the necessity to utilize multiple different biomarkers for early OSCC detection with salivary biomarkers. Additional studies have reported new transcriptomic biomarkers for OSCC that may increase the feasibility of utilizing saliva for discriminatory OSCC detection in future combination biomarker studies (Table 2).

MMP transcripts have been found to be over-expressed in OSCC patients [66,67], and *MMP-1* and *MMP-9* have been associated with the progression of dysplasia to cancer [68]. *MMP-1* transcript levels in saliva have been shown to be higher in OSCC patients than controls [51,69].

Micro-RNAs (miRNAs) are regarded as important regulators of mRNA and protein expression and are predicted to regulate the expression of almost one-third of all human transcripts [70,71]. MiRNAs can function as either tumor suppressors or oncogenes depending on their target transcripts. Numerous studies have described the potential of miRNAs as cancer biomarkers for oral cancer [72,73]. In a recently published study, approximately 50 miRNAs were detected in saliva using reverse transcriptase-preamplification-quantitative PCR. In addition, significantly lower levels of *miR-125a* and *mi-R200a* were found in the saliva from 50 OSCC patients compared to 50 healthy control subjects [74**,75]. Salivary *miR-31* increased significantly in patients with OSCC at all stages, and then decreased after the cancer had been excised. Along with the increased *miR-31* in plasma, saliva and blood diagnostics may also lead to powerful OSCC biomarker prediction and disease progression [72].

MICROBIOMICS

Bacterial infections were previously connected to malignancies because of their ability to promote chronic inflammation [76,77]. A recent study compared the microbial species from the tumor and non-tumor tissues of patients with OSCC using denaturing gradient gel electrophoresis (DGGE), cloning, and sequencing. *Peptostreptococcus stomatis*, *Streptococcus gordonii*, and six other named cultivable bacterial species were found to be highly associated with tumor sites, whereas *Granulicatella adiacens*, a known factor in endocarditis, was prevalent at non-tumor sites. The same microbial diversity was found in the saliva of patients with OSCC. Noting the changes in oral microbiota, species colonization may aid in determining the evolution of pre-cancerous lesions into OSCC malignancies, for use as a diagnostic tool. Further research of the most prevalent species identified in tumor tissues is needed to formulate their role in cancer development [78,79*].

Human papilloma virus (HPV) is associated with oropharyngeal squamous cell cancers, and 60% in the US have been shown to be positive for HPV type 16. Current tests for HPV detection by saliva are available utilizing polymerase chain reaction (PCR) [80]. As early diagnosis is critical for OSCC, further advancements in creating point of care technologies for HPV associated OSCC detection are likely to occur.

METHYLOMICS

Methylation has been suggested to be an early event in oral carcinogenesis. In OSCC, hypermethylated genes have been associated with alterations in proliferation, DNA repair, apoptosis, cell-cell adhesion, and angiogenesis, suggesting them as potential biomarkers for oral cancer, [81, 82**]. A genome-wide DNA methylation platform was utilized to uncover differentially methylated genes in saliva from OSCC patients and normal controls. This phase I Biomarker Development Trial identified Homeobox protein Hox-A9 (*HOXA9)* and nidogen 2 *(NID2)* as methylated genes in OSCC patients. Thus, promoter methylation of genes in saliva may serve as potential biomarkers for the early detection of OSCC [83].

METABOLOMICS

Metabolomics is a measure of all intracellular metabolites and is a potent tool for understanding cellular function [84,85]. Metabolomics-based technology is emerging for the identification of disease-associated salivary analytes. Sugimoto et al. published a novel study in which they compared the salivary metabolic profiles of patients with oral cancer and healthy controls. Twenty-eight metabolites, including pyrroline, choline, and valine, were found to be discriminatory between subjects with oral cancer and healthy controls [34].

Cancer is often correlated with an altered glucose metabolism. Most cancer cells have a high rate of aerobic glycolysis, also referred to as the Warburg effect, for the generation of ATP, resulting in increased lactate production. A research group from University of Michigan, recently performed global metabolic profiling of metabolites in head and neck squamous cell carcinoma subjects. The metabolites associated with malignant transformation of head and neck neoplasia, could be related to the Warburg effect [86]. Further investigation in the metabolomics and the biological importance in oral cancer is needed.

EXOSOMES AND CIRCULATING BIOMARKERS

Lately, interest in the biology of extracellular vesicles has increased greatly. MiRNA is one of the most commonly identified genetic materials in exosomes. Exosomes are regarded as a novel mechanism by which cancer cells and virally-infected cells can regulate their micro-environment. Exosomes and microvesicles (MVs) are nanometer-sized membranous vesicles secreted from many cell types into their surrounding extracellular space and body fluids [87]. Exosomes and MVs have also been found in saliva [88,89]. Studies have examined the biological activity and molecular functions of MVs in oral cancer progression [28,90], but biofluids have different properties and contain a wide range of exosomes and MVs secreted from various cell types. The cell culture supernatant is an ideal model for exosome purification. However, the viscosity and cellular contamination of WS make it a less than ideal medium for exosome isolation [28,90].

Concluding remarks and areas for further research

Saliva has become a more and more attractive tool due to advances in novel technology and its potential for the surveillance of general health and disease. Sensitive technology is needed to detect biomarkers in low quantities in order for saliva to be an effective diagnostic medium [91]. Engineers are now pioneering and advancing the development of an electrochemical biosensor capable of identifying salivary biomarkers with high sensitivity and specificity. An oral cancer study found that the sensor had approximately 90% specificity and sensitivity for *IL-8* mRNA and IL-8 protein with a limit of detection of 3.9 fM and 7.4 pg/mL, respectively [92]. The final product, called the Oral Fluid NanoSensor Test (OFNASET), is an automated and easy-to-use system able to quickly and simultaneously detect multiple salivary protein and nucleic acid targets for the determination of various diseases. This point-of-care electrochemical sensor system can be used in the office of a dentist or another health care provider to deliver quick disease screening results [93–95].

Furthermore, to allow for an accurate assessment of biomarkers, researchers should follow the Prospective Specimen Collection, Retrospective Blinded Evaluation (PRoBE) design in which biological specimens are collected from a cohort population that resembles the population where the biomarker will be used. For example, a study to determine the sensitivity and specificity of a particular marker for OSCC would need to be tested in a population at high risk for developing the cancer. The second part of the PRoBE design ensures that subject diagnoses are blinded in order to obtain unbiased data on biomarker specificity and sensitivity [96]. Currently, a nationwide PRoBE design study for OSCC saliva biomarkers is underway that meets the guidelines of the NIH. Dr. David Wong and his team at the University of California, Los Angeles, are enrolling subjects to validate salivary oral cancer biomarkers and determine the performance of already discovered transcriptomic and proteomic panels. OSCC is multi-factorial with a heterogenic pathogenesis; thus, one single biomarker may not be able to discriminate between OSCC and controls. Multiple biomarker candidates are needed for high accuracy and sensitivity in detecting OSCC. A combination of the transcriptomic and proteomic salivary biomarkers was tested in an independent cohort of OSCC patients from Serbia. Three protein markers

(IL-8, IL-1β, and M2BP) and four mRNA markers (*IL-8, IL-1β, SAT1*, and *S100P*) were elevated and discriminatory for late-stage OSCC [64**,65**, 97].

Early detection of premalignant lesions is associated with improved survival in OSCC patients. Unfortunately, most OSCC cases are detected when the cancer has developed into the advanced stages. Biopsies have several disadvantages, including relatively high costs, inaccurate diagnoses due to difficulties sampling tissue, and patient discomfort with the procedure. Reliable early stage diagnostic markers for OSCC are currently lacking. Sensitive and specific biomarkers for OSCC will be helpful in screening high-risk patients and to follow-up patients for early signs of recurrence. Furthermore, salivary biomarkers can be used between biopsies to assist in monitoring the disease status of dysplasia patients. Although extensive and thorough biomarker validation is essential before any biomarker candidates can be tailored for clinical use, salivary diagnostics for OSCC are very promising due to the direct contact of saliva with premalignant or malignant lesions.

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* Of importance

** Of major importance

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Table 1

Recent publications of proteomic biomarkers for Oral squamous cell carcinoma (OSCC)

Biomarker	Function	Reference	Sensitivity (%)/Specificity (%)/AUC
Interleukin-1 alpha (IL-1α)	Protein	[54]	NR/NR/NR (°p<0.0001)
Interleukin-1 beta (IL-1β)	Protein	[55], [65]	75/80/0.77
Interleukin 6 (IL-6)	Protein	[54]	NR/NR/NR (°p<0.0001)
Interleukin 8 (IL-8)	Protein	[54], [55], [65]	75/80/.80
Tumor necrosis factor alpha (TNF-α)	Protein	[54]	NR/NR/NR (°p<0.0001)
Vascular endothelial growth factor A (VEGF-A)	Protein	[54]	NR/NR/NR (°p<0.0001)
Matrix metalloproteinase-1 (MMP-1)	Protein	[51]	NR/NR/0.845
Matrix metalloproteinase-3 (MMP-3)	Protein	[51]	NR/NR/0.8766
Matrix metalloproteinase 9 (MMP-9)	Protein	[52]	100/79/NR
Carbonyls	Products of Protein Oxidation	[52]	90/80/NR
Mammary serine protease inhibitor (Maspin)	Protein	[52]	100/100/NR
Cyclin D1 (CycD1)	Protein	[52]	100/100/NR
Transferrin	Protein	[45], [57]	NR, 100/95/.91 – .95*
Total sialic acid	Protein	[62]	61/44/0.653
α-L-fucosidase	Protein	[62]	69/48/0.696
Actin	Protein	[56]	69/48/0.696
Myosin	Protein	[56]	100/75/NR
Basic fibroblast growth factor (bFGF)	Protein	[61]	67/83/NR
Combination:			
Myeloid-related protein 14 (MRP14) + Profilin+ CD59 + Catalase + Mac-2-binding protein (M2BP)	Protein Combination	[55]	90/83/0.93

°p-value if no other parameters reported

*for different OSCC stages

Table 2

Recent publications of transcriptomic biomarkers for Oral squamous cell carcinoma (OSCC)

Biomarker	Function	Reference	Sensitivit (%)/Specificity (%)/AUC
Matrix metalloproteinase-1 *(MMP1)*	mRNA	[51]	NR/NR/0.984
Interleukin 8 *(IL-8)*	mRNA	[65]	66/80/0.77
S100 calcium binding protein P *(S100P)*	mRNA	[65]	54/88/0.71
Spermidine/spermine N1-acetyltransferase 1 *(SAT1)*	mRNA	[65]	54/82/0.70
Ornithine decarboxylase antizyme 1 *(OAZ1)*	mRNA	[65]	40/92/0.60
Interleukin-1 beta (*IL-1β*)	mRNA	[65]	83/76/0.83
Dual specificity phosphatase 1 (DUSP1)	mRNA	[65]	14/98/0.41
miR-200a	miRNA	[74]	NR/NR/0.65
miR-125a	miRNA	[74]	NR/NR/0.62
miR-31i	miRNA	[72]	80/68/0.82
Combinations:			
IL1β + SAT1 + DUSP1	mRNA	[65]	89/78/0.86
IL8 + IL1- β + SAT + OAZ1	mRNA	[64]	79/77/0.86