IRAQI Academic Scientific Journals
Samarra Journal of Pure and Applied Science
SJPAS
مجلة سامراء للعلوم الصرفة والتطبيقية
www.sjpas.com
p ISSN: 2663-7405
e ISSN: 2789-6838

Saliva miRNAs could be used as potential diagnostic biomarkers

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https://doi.org/10.54153/sjpas.2022.v4i2.379

Article Information
Received: 10/04/2022
Accepted: 09/05/2022
Keywords:
Saliva,miRNA,cancer, periodontitis
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Abstract

microRNA are small, noncoding RNA that mediate translational repression or mRNA breakdown of spesific gene,also acting as important regulators of a variety of biological activities. Salivary miRNAs are gaining popularity as noninvasive diagnostic techniques for cancer and other systemic illnesses. Oral epithelia and also bacteria in oral cavity are two main source of salivary RNAs. Recent findings imply that disorder such as oral cancer and other disease can be diagnosed using identified salivary RNAs biomarkers.

Introduction:

MicroRNAs are a form of noncoding single-stranded RNA discovered in *C. Elegans* and then found to be conserved in a wide range of animal types including human binge [1]. The regulation of miRNA to gene expression has opened the door for new treatment techniques, the miRNAs can be regulating the influence of different types of mutation and gene dysfunction [2].

The method by which miRNAs are generated is known as miRNA synthesis. In this procedure AS Figure (1) show miRNAs are transcribed as a primary transcript (pri-miRNAs) with 5 cap and 3 poly A tail, and maturation process of the pri-miRNAs is a two- steps procedure that starts with a pri- miRNA production procedure by Drosha ribonuclease into 7simple nucleotides RNA molecules with stem loop - structures. The Exportin-5 receptor take the pre miRNA from the nucleus to the cytoplasm and they are cleaved to the short and double-stranded miRNA by an endoribonuclease Dicer (helicase with RNase motif) [1], A helicase enzyme unravels the miRNA duplex into mature miRNA individual strand which are then integrated into the RNA influenced silencing complex RISC, which starts the RNA-based gene suppression process [3].

miRNA controls gene expression after transcription by binding to mRNAs, inhibiting their translation and shortening their half-life [4]. miRNAs control gene expression by interacting with the UTRs of spesific mRNA, resulting in mRNA breakdown or translation suppression [5].

Extracellularly, miRNAs can be secreted, including into the bloodstream, where they are hypothesized to be a cell-to-cell contact mechanism [6]. miRNAs are abundant in serum and plasma, and they have the ability to be used as diagnostic biomarkers for a number of physiological and pathological conditions, like cancer, infection, and heart dysfunction, among many others [7].

The salivary transcriptome, which comprises over three thousand RNA types, including miRNA was secreted and salivary miRNA as medical biomarker are being used to diagnose systemic illnesses and mouth cancer [8].

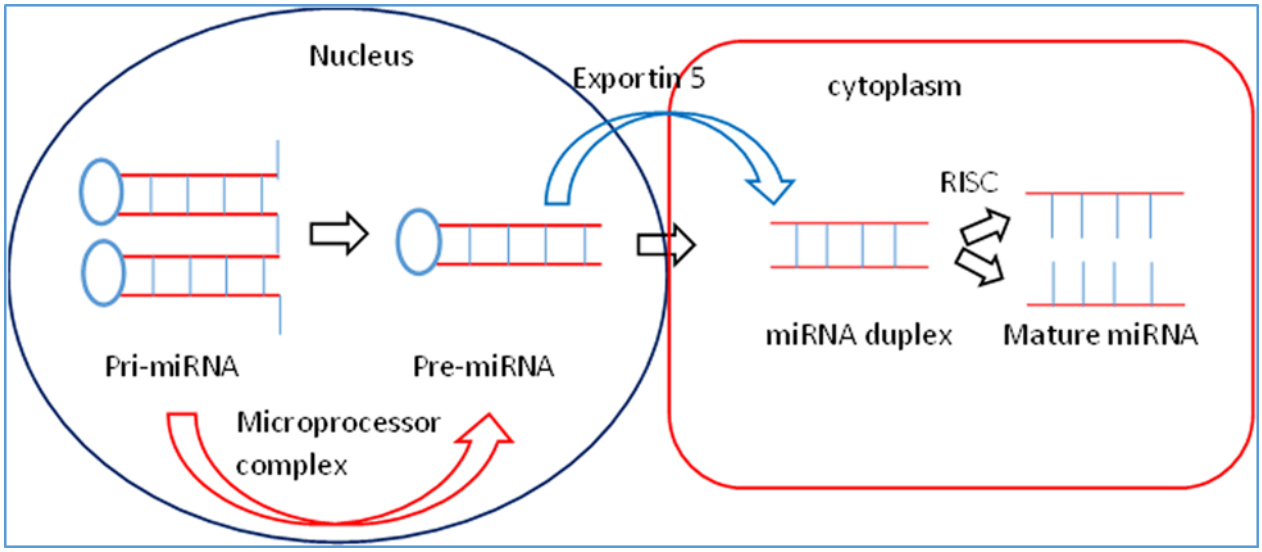

Fig (1): steps of microRNAs biogenesis [2].

There is a significant relation between miRNA abnormality, epigenetic alteration, and disease progression. About fifty percent of the miRNA gene have (cytosine phosphate guanine) island and the DNA methylation process can be modified these island, also, miRNA gene expression may vary in illness condition versus to normal condition, so the miRNAs have a specific approach of methylation in each disease. increase and decrease of methylation rate of miRNAs genes promoter have a direct effect on progression of a many pathogenic conditions [2].

Human Saliva

Saliva is a mixture of fluid and cellular components. The (3) three principal cell types that coexist alongside many bacterial cells in human saliva are leukocytes, epithelial cells, and erythrocytes [9]. The gingival sulcus, and also the submandibular, sublingual, parotid, and minor salivary glands, produce saliva, which would be a mixture of fluids. All biological liquids have unique chemico-physical properties, as is well known. Saliva comprises a wide variety of chemicals generated by microorganisms and exogenous sources. Sex, age, circadian rhythm, medicine, nutrition, and environmental exposures may all have an effect on the quality and amount of substances released by the same individual [10].

Saliva sampling can be done by people who aren't professionals. Because it is noninvasiveness, it may be used on the elderly, children and for many samplings [11]. The

measurement of protein or the RNA molecule in oral saliva was focus of many diagnostic biomarker investigations. Because the diseases detected by salivary biomarkers are frequently systemic diseases, identifying circulating RNA molecules or protein that may have been produced by disease relevant cells such as tumor cells is of great interest. Such molecules are They are frequently captured in cell free saliva (CFS) and are found outside of the cells in saliva. When compared to full-length mRNA in the cellular compartment, most salivary RNAs are severely damaged, demonstrating the existence of an RNA degrading enzyme in saliva and many other body fluids [12].

Salivary microRNAs

The first paper to use the phrase (salivary RNA) was Evidence for Two Metabolically Particular types of ribonucleic Acid with in chromatin and the nucleoli [13]. In 2012, Wong group published first worldwide analysis of the individual salivary transcriptome by use high throughput RNA sequencing. Saliva has a diverse range of RNA types, as per the researchers. In- coding and noncoding RNAs, over 4,000 different RNA molecules, including miRNAs, have indeed been found. The RNA composition of human saliva was discovered to be quite diverse, which could be further studied in future diagnostic biomedical research [14].

Saliva give exact fact about human oral cavity as it participates to its environments making the human salivary RNA a useful tool for identifying numerous disorders linked with the oral cavity. Because of the features of salivary RNA, it may be able to convey information about diseases that occur in other sections of the body. Saliva has RNA indicator for pancreatic cancer, breast cancer, and biomarker that may be beneficial in identifying the type 2 diabetes. However, current state of knowledge regarding miRNA as bio markers is insufficient to practical application [11].

When compared to other salivary diagnostic biomarkers such as proteins, DNA, mRNAs, and bacterial products, miRNAs in saliva have a number of advantages as biomarkers. Apart from miRNA's specific role miRNAs are relatively stable in human saliva as a post-transcriptional regulator, and similarity of miRNA profile in saliva and the fluid makes them readily available as disease markers for many diseases in humans [15].

It's difficult to isolate and deal with RNA for its instability. The temperature of the samples must be kept steady, and the characteristics of saliva RNA must be regarded when estimating the results. Saliva contains virus-derived RNA, even though its stability is still to be established. Microorganisms in the oral cavity produce endonucleases and exonucleases, which change the safety and sequence of salivary RNA [16].

Salivary microRNAs and cancers

Oral cancer, most frequently oral squamous cell carcinoma OSCC is the sixth most frequent cause of tumor Deaths, consider for almost 90% of all oral cancer cases. The five - year survival rate is around to 8 % if OSCC is discovered at an early stage tumor size T-1. If OSCC is discovered at a later stage, such as tumor size T-3 - T-4, the five-year survivorship rate goes down to be between (20–40%), implying that early diagnosis strategies are essential for improving long-term patient survival. Previously, OSCC was diagnosed using mRNA, DNA, and protein isolated from saliva [17]. Overexpression of specific miRNAs may result in tumor suppressor gene (TSG) downregulation, while under expression of other miRNAs may result

in oncogene overexpression [18]. Uncontrolled expression of some miRNAs has been shown to alter cell development and can act as tumor suppressors genes or oncogene in a variety of malignancies. miRNA, has been shown to affect cell apoptosis, proliferation and even in OSCC patients who are resistant to chemotherapy DNA methylation has also been shown to epigenetically regulate miRNAs, in OSCC patients. Since cancer cells express miRNAs differently cells, they have distinct expression profiles [19].

miRNAs, are used as disease markers for cancer prognosis because they are reasonably constant both in circulation and tissues [20]. Many earlier researches on The main focus of miRNA profiling was on the differential gene expression of miRNAs in the bodies. Fluid like a blood, saliva, serum, and plasma from OSCC patients, and so many previous research on miRNA characterization focused primarily on altered gene expression of miRNAs in blood and the other body fluid like saliva, serum, and plasma from OSCC patients population as show in table 1 [21].

Park and colleagues looked investigated how many different types of miRNAs were generated in the saliva of OSCC patients and normal people. In (OSCC) patient, miR-125a and miR-200a were shown to be lower than in controls $p \leq 0.05$ [19]. Nasopharyngeal neoplasia NPC is a cancer of the nasopharyngeal epithelial that is exceedingly rare around the world, with prevalence rate of the less than 1 per 100,000-person year [22]. By up - regulating its targets mRNAs that express extracellular proteins, (miR-29c) suppression causes increased migration and invasion of NPC cells [23].

Table 1: Different expressed miRNAs in oral carcinoma (up and down regulation) [24].

Deregulation	miRNA
Down-regulated	miR-5580-3p, miR-617, miR-6510-3p, miR-6746-5p, miR-6801-5p, miR-4521, miR-199b-5p, miR-509-3p, miR-29c-3p, miR-486-3p, miR-6762-5p, miR-6838-5p, miR-99a-3p, miR-7641, miR-1224-5p, miR-6868-5p, miR486-5p, miR-3659, miR-451a, miR-19a-3p, miR-138-5p, miR-139, miR-1295a, miR874-5p, miR-514b-5p, miR-758-3p, miR-497-5p, miR-30c-1-3p, miR-204-5p, miR-99a-5p, miR-6756-5p, miR-139-5p, miR-6086, miR-101-3p, miR-494-3p, miR-27b-3p, miR-7162-3p, miR-664a-3p,miR-509-5p, miR-3911, miR-7977, miR-23b-5p.
Up-regulated	MiR-6877-3p, miR-1908-5p, miR-3780b-5p, miR-3180, miR-6738-5p, miR-1237-5p, miR-4443, miR-6870-5p, miR-3180-3p, miR-4442, miR-4788, miR-548w, miR-6126, miR-4495, miR-6805-5p, miR6765-5p, miR-4706, miR-149-3p, miR6754-3p, miR7846-3p, miR-8072, miR-146b-5p, miR-135a-3p, miR-5585-3p, miR-5196-5p, miR-6753-3p, miR-885-3p, miR-3175, miR-3937, miR3934-5p, miR-663b, miR-1290, miR-7111-5p, miR6825-5p, miR-4417.

In vitro, overe gene xpression of miR-378 in NPC tissues decreases the production of TOB2(antiproliferative cell protein) a potential TSG, and improves colony formation, proliferation, migration and cell invasion substantially [25]. The (MiR-155, miR-26a, miR-98, miR-205, miR-200a/b, and miR-216b) regulate a number of significant processes, include Epithelial Mesenchymal Transition (EMT) gene and signal transduction pathways like phosphatidylinositol 3-kinase [26].

In a recent study, patients having malignancy and benign pancreatic tumors were contrasted to healthy controls. When analyzing the cancer group to the other groups, the authors discovered a significant decline in miR-3679-5p expression and an elevation in miR-

940 production in the cancer group, demonstrating that mouth miRNA can be used to detect pancreas cancer at an early stage [27].

Different kinds of miRNAs (miR-1233, miR-296-5p, miR-1267, miR-211, miR-1825, and miR-425-5p) were found to be considerably highly expressed in saliva from Parotid Gland Cancer patients when compared to controls [28]. The microRNAs (miR-374, miR-132, miR-519b-3p, miR-222, miR-15b let-7g, miR-223, miR-140-5p, and miR-30a-3p) were confirmed in a separate group of samples that shows very high salivary level in patient with a malignancy parotid glands tumors [29].

Using a specifically made bio sensing nanographene oxide systems that uses 2 differents wavelengths to detect miR-21 & miR-141 from human bodies fluids such as urine, blood, and saliva, Hizir M.S. and his collaborators have evidenced simultaneous determination of endogenous and exogenous miR-141 and miR-21 from contact with bodily fluids such as urine, blood, and saliva [30].Nasopharyngeal carcinoma of the nasopharyngeal epithelial tissue that is exceedingly rare in most parts of the world, with prevalence rate of fewer than one per 100,000 person years [31].Salivary miR-3679-3p, miR-574-5p, miR-6131 and miR-205-5p expression was elevated in NPC patients. 47 miRNAs, notably miR-575, miR-30b-3p, and miR-650, revealed a reduction in expression in NPC patients when compared to controls [32].

Salivary microRNAs and systemic disease

miRNA, deregulation has been linked to various of mechanisms in a range of systemic diseases. By unusual expression of several miRNAs situated in the affected parts, accompanied by down regulation of their down - stream mRNA targets, hereditary alterations such as genomic amplifications, chromosomal aberrations, deletions, and point mutations play a vital role in disease initiation and/or progression [33].

Many medical biomarker studies have focused on quantifying proteins and RNA molecules in saliva because of disorders detected by salivary diagnostic biomarkers are usually systemic. Specific miRNA and mRNA molecule were shown to be extremely stable, due to protein or exosome complexe that protect them [34].

In a medical trial on a chronic immune disorder (Sjögren's Syndrome), the researchers discovered distinct miRNA gene expression profiles in the minor salivary gland of Sjögren's Syndrome is a condition compare to the control subjects [35]. Salivary miRNAs appear to be holding a great deal of promise for both early diagnosis and understanding the pathophysiology of neurological and mental diseases [36].

Investigating the involvement of miRNAs in inflammatory processes could lead to a better knowledge of immunological homeostasis as well as a novel therapeutic technique for treating inflammation. Periodontitis is a persistent periodontal inflammation that causes the loss of bone and connective tissue that supports the teeth. The balance of interactions between the microbial challenge and the human immune inflammatory responses is required for periodontitis to develop [37].

miRNA-146b, miRNA-146a, and miRNA-155 concentrations were higher different in normal and irritated gingiva. This large differences in miRNA profiles between periodontitis and normal gingiva suggests a strong linkage between miRNAs and the periodontitis diseases [38].

miRNA-204 level were lower in gastritis patients' blood, ulcer tissue and saliva sample from *Helicobacter pylori* than in control. These findings suggest that the miR-204 may play a critical role in the regulation of *Helicobacter pylori* associated with gastroenteritis by targeting the Matrix metallopeptidase 9 (MMP-9) mRNA [39]. Salivary concentrations of miR-31 were found to be very high in ulcerative colitis patient than in healthy individuals. Many aggressive diseases have been related to miR-31ulcerative colitis, Charon's disease [40].

Restriction use of the salivary microRNAs

Saliva collection is generally less complicated than blood collection and does not necessitate the use of professional people. However, the components of saliva may not be highly stable, which could restrict their utility. One of the major drawbacks of employing human salivary RNA for diagnostic biomarkers, in addition to the other components in saliva, is its stability [41].

One challenge is the impartial separation of long and short RNA from saliva sample. Despite advances in molecular biology and performance is achieved technologies that enable scientists to evaluate miRNA expression in body fluid like as plasma, serum, and saliva on a huge scale, the lack of adequate endogenous control for salivary miRNA normalizing continues to be an issue [42].

It is impossible to apply the approach that is typically used with blood without optimizing it for saliva cause When working with the salivary molecule like RNAs and miRNAs the method should be has high detection sensibility rat to get extremely reliable results [43].

Studying the biogenesis pathways of salivary non - coding RNAs is the framework for determining what extend ncRNA production could show a patient ’s health conditions. The transcriptome in saliva has indeed been widely researched. Microbial RNA, which must be separated from human-derived RNA, is also available as an oral. It could also include food residues, some of which could bias the sample analytical results [44].

Conclusion

The combination of novel diagnostic procedures and saliva collection will allow larger and follow up research to be undertaken at cheaper value compare to use of blood or tissues specimens. There are several obstacles in this sector, the majority of which are common to the use of any bodily fluid in biomedical research. The isolation of small or large RNA molecule from saliva specimens is one of the most difficult challenges.

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www.sjpas.com
p ISSN: 2663-7405
e ISSN: 2789-6838

الاحماض النووية الرايبوزية الصغرى اللعابية وإمكانية استخدامها كمعلم تشخيصي

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معلومات البحث:
تأريخ الاستلام: 2022/04/10
تأريخ القبــول: 2022/05/09
الكلمات المفتاحية:
السرطان, التهاب اللثة, اللعاب, حماض نووية رايبوزية صغرى

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الخلاصة:

الاحماض النووية الرايبوزية الصغرى عبارة عن جزيئات صغيرة غير مشفرة تتوسط في قمع أو تثبيط mRNA لجين معين، ويعمل أيضًا كمنظم مهم لمجموعة متنوعة من الأنشطة البيولوجية. تكتسب miRNAs اللعابية أهمية باعتبارها تقنيات تشخيصية للسرطان وأمراض جهازية أخرى.و تعتبر ظهارة الفم والبكتيريا الموجودة في الفم مصدرين رئيسيين للحمض النووي الريبي الصغير اللعابي. تشير النتائج الحديثة إلى أنه يمكن تشخيص اضطرابات أخرى باستخدام المؤشرات الحيوية للاحماض النووية الرايبوزية الصغرى اللعابية.