Piva et al., Dent Health Curr Res 2017, 3:1
DOI: 10.4172/2470-0886.1000e105

Dental Health: Current Research

Editorial A SCITECHNOL JOURNAL

The Importance of Saliva for Biomolecule Sampling

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Abstract

The oral compartment is an interesting source of biomolecules that could support or even replace blood sampling. Although having the evident advantage of minimal invasiveness, saliva employment for biomarker recovery did not gain ground because it was believed that only few blood molecules could be recovered from saliva. Recent evidence shows that, not only most blood molecules can be found in saliva but also that in saliva there are molecules not present in blood. Moreover saliva contains molecules coming from distant sites such as exosomes. Here we discuss recent evidence that makes saliva promising for biomarker sampling.

Keywords

Saliva; Exosomes; Biomarkers; MicroRNA

Introduction

The assessment of biomolecules from salivary instead of blood compartment to detect systemic diseases would be advantageous thanks to its non-invasiveness and the chance to perform an auto sampling by the patient. Recently the saliva is emerging as a promising source of biological information because early evidence shows that, like blood, it contains substances coming also from distant cells. Moreover saliva could give even more information than blood since in the former there are some specific biomolecules not present in blood. In particular, the high throughput omics technologies indicate promising candidate disease-specific biomarkers in blood but only recently researchers have begun to assess if these biomarkers are present and are not altered also in saliva. Saliva, like blood contains a large amount of substances, mainly proteins, DNA and RNA.

Salivary proteins

Saliva is interesting source of biomarkers because it is rich in proteins. In fact, high resolution mass spectrometry analysis revealed that saliva contains more than 1200 proteins [1] and these add to the previously known ones thus giving about 3500 proteins. However saliva could give information which is different from plasma since some salivary proteins are not found in plasma. This is expected since saliva contains secretions from the salivary glands, an inflammatory exudate named gingival crevicular fluid (GCF), nasal and bronchial secretions, micro-organism enzymes and degradation products.

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Received: February 17, 2017 **Accepted:** February 20, 2017 **Published:** February 26, 2017

Salivary DNA

For example, saliva contains human and microbial genomic DNA that are more than enough to perform polymerase chain reaction (PCR) assays [2], so they can be used to assess human sequence variations and epigenetic marks. However it should be taken into account that salivary human DNA derives from leucocytes (granulocytes, lymphocytes, and monocytes) and dead cells such as exfoliated epithelial cells. Salivary DNA can also reveal the presence of Helicobacter pylori [3] or hepatitis B virus [4,5] and also virus level in saliva is higher than in blood samples.

Salivary RNA

Also mRNA and noncoding RNA can be retrieved from saliva and their quantification can give information on different diseases. For example, salivary RNA of CCNI, EGFR, FGF19, FRS2, and GREB1 genes could detect lung cancer patients with good sensitivity (93.75%) and specificity (82.81%) [6]. Salivary RNA of KRAS, MBD3L2, ACRV1, and DPM1 distinguished pancreatic cancer patients from healthy subjects or with chronic pancreatitis with good sensitivity (90.0%) and specificity (95.0%). Salivary RNA of CSTA, TPT1, IGF2BP1, GRM1, GRIK1, H6PD, MDM4, S100A8 and salivary CA6 protein discriminated breast cancer patients from healthy subjects with good sensitivity (83%) and specificity (97%) [7].

Another interesting challenge is represented by the study of thousands of stable circular RNAs (circRNAs) of which about 400 were identified in saliva [8] and could represent potential biomarkers for diseases [9]. Although the role of circRNAs is largely unknown, at least two circRNAs act as miRNA sponges [10].

Also salivary microRNAs are related to diseases and these molecules are more attractive than longer RNAs thanks to their stability. In fact, it was shown that miRNA-196a could be a potential diagnostic biomarker for oesophageal squamous cell carcinoma [11], miR-21 could be a biomarker for colorectal cancer [12], miR-139-5p could be a potential biomarker for early tongue squamous cell carcinoma detection [13]. Salivary miR-21, miR23a, miR-23b and miR-29c were significantly up-regulated in patients with pancreatic cancer compared to control patients and miR-216 could discriminate pancreatic cancer from pancreatitis [14].

Curiously, by high-throughput RNA sequencing it was shown that miR-223–3p, miR-148a-3p, miR-24-3p, miR-21-5p and miR-26a-5p are the most abundant miRNA in saliva of healthy individuals [15] but plasma/serum miR-223 are closely associated with the tumorigenesis and metastasis of different carcinoma. In fact, plasma/serum levels of miR-223 and miR-21 were significantly higher in gastric cancer patients [16] and hepatocellular carcinoma or chronic hepatitis [17].

However, the majority of miRNAs in saliva are not free-circulating but concentrated in exosomes [18].

Salivary exosomes

Exosomes are 40-120 nm lipid microvesicles released from all cells, circulating in all body fluids and specifically merged by recipient cells. They gained interest because they carry RNA, DNA and proteins

that constitute messengers able to condition/affect the behaviour of the receiving cell. Moreover exosomes have a good survival thanks to their membranes that effectively protect the content. In body fluids exosomes released by all cells can be recovered but it would be useful to know for each exosome its originating and addressee cell. To establish/recover this information would be possible, in fact, the pattern of exosome membrane proteins depends on the releasing and addressee cell. In other words, all the different protein patterns of exosomes constitute the words of a language that researchers are decoding, that is, they are establishing the correspondences/links between a specific membrane protein of an exosome and its origin and destination. Some discoveries are outstanding and encouraging as, for example, the finding that exosomes exposing Glypican-1 protein specifically come from pancreatic cancer cells [19]. Besides from blood, exosomes can be recovered also from saliva and more abundantly from buccal swabs. Surprisingly, to our knowledge, it has not yet been assessed if exosomes carrying Glipican-1 are present also in saliva.

In order to validate/consolidate exosome sampling in saliva, it will be interesting to verify which blood exosomes can also be found in saliva. The evidence that blood exosomes can reach the oral cavity means that it is a valid gateway to administer exosome-based therapies. Actually, early evidence demonstrates that exosomes of tumours distant from the oral cavity can reach the oral compartment and here they are functionally active. For example, in an animal model, salivary exosomes from pancreatic ductal adenocarcinoma (PDAC) alter exosomal production of salivary glands that, in turn, alter immune surveillance interacting with NK cells (Figure 1) [20].

Not always the attempts of translation results from blood to saliva succeed. For example, miR-1246, miR-3976, miR-4306, and miR-4644 from serum exosomes are shown to be biomarkers for pancreatic cancer [21] but, unfortunately, the same results are not obtained sampling the same miRNAs in salivary exosomes. In particular, only miR-1246 and miR-4644 maintained their value as biomarkers but with sensitivity slightly lower than the same miRNAs in blood [22].

However microRNA from salivary exosomes are giving promising results, in fact, saliva exosome level of miR-4484, that is involved in immune responses to pathological stimuli, could be a biomarker of oral lichen planus [23], miR-24-3p from salivary exosomes could be a candidate aging biomarker [24].

Also piwi-interacting RNA (piRNA) is abundant in saliva and at least two piRNAs are localized in salivary exosomes [25]. PiRNAs are thought to function forming complexes with PIWI proteins, thus promoting transposon silencing and transcriptional regulation [26].

Perspectives

The assessment of biomolecules from saliva is promising, not only for its minimal invasiveness but also because it could be even more advantageous than blood in terms of uniqueness of recovered molecules. Of course, the translation of biomarkers from blood to saliva and the identification of new biomarkers among molecules present uniquely in saliva is challenging. Researchers will have to take into account bacterial interfering in saliva and sample storage. However salivary exosomes are important because they carry intact information from distant sites; moreover exosome sampling will be even more important when we will be able to determine their cell of origin.

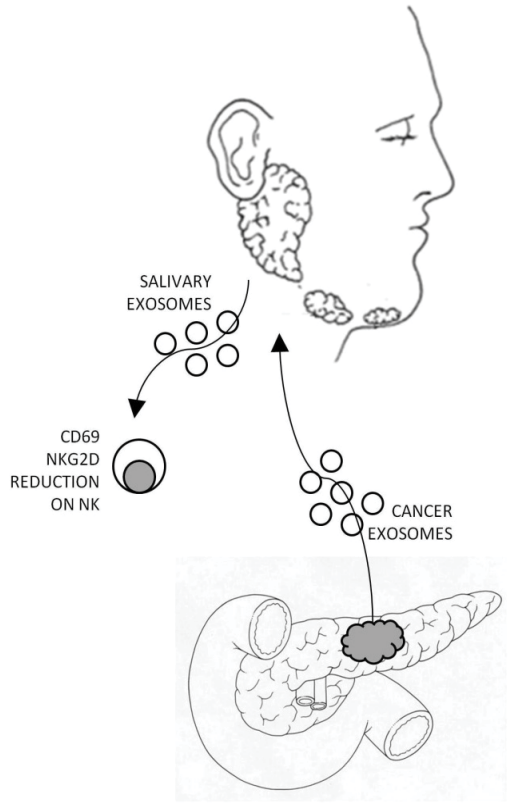

Figure 1: In a mouse model, it was shown that pancreatic cancer-derived exosomes reach salivary gland cells altering their exosome release. Salivary exosomes can be taken up by NK cells causing lower activation, as indicated by lower CD69 (Early activation antigen CD69) and NKG2D (NK killer group 2D receptor) expression. Although the figure refers to humans, its validity in humans has not yet been demonstrated.

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