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Highlights

Saliva biomarkers in neurological disorders: A "spitting image" of brain health?

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ABSTRACT

In this issue of the *Biomedical Journal*, we learn how biomarkers in saliva may be able to provide insight into the health of the brain and the central nervous system. We also discover how computational modeling can help to identify potential epitopes for vaccine development against Chlamydia, the world's most common sexually transmitted infection.

Spotlight on reviews

Saliva biomarkers in neurological disorders: a "spitting image" of brain health?

The key to managing effectively the symptoms and limiting the progression of neurological diseases lies in early diagnosis. Yet, diagnosis remains challenging due to the complicated causes and manifestations of these diseases, and may involve invasive and painful tests like lumbar puncture. In this issue of the *Biomedical Journal*, Farah et al. [1] review a multitude of studies revealing that saliva may be a valuable source of biomarkers to facilitate early diagnosis of neurological diseases.

Saliva may appear pretty uninteresting but it is actually a complex liquid composed of various enzymes, hormones, antibodies, antimicrobial constituents, and growth factors [2]. All in all, your spit contains more than 2000 proteins, 27% of which are found in blood [3] and actually enter saliva directly from blood by passing through the space between cells [4]. Thus, saliva is functionally equivalent to serum in terms of reflecting the health status of the human body. It is also a lot easier to sample, with subjects being able to produce an ample supply, pain free and on demand (the average human actually produces somewhere between 1 and 1.5 L of the stuff every day). The feasibility of using saliva as a source of diagnostic biomarkers, "salivary diagnostics", has been examined for a whole host of diseases, including oral diseases, cancer, HIV, diabetes and cardiovascular diseases (reviewed in Ref. [5]).

But what can our saliva tell us about the health of our brain and the central nervous system? The answer, according to Farah et al. [1], fortunately appears to be quite a lot [Fig. 1]. Remarkably, even the proteins implicated in the pathological development of certain neurological diseases are capable of being detected in spit, and can hold vital information about diagnostics or disease status. Take for example alpha-synuclein (α-syn), the main constituent of Lewy bodies, which are the toxic protein clumps thought to lead the loss of dopaminergic and serotoninergic neurons in Parkinson's

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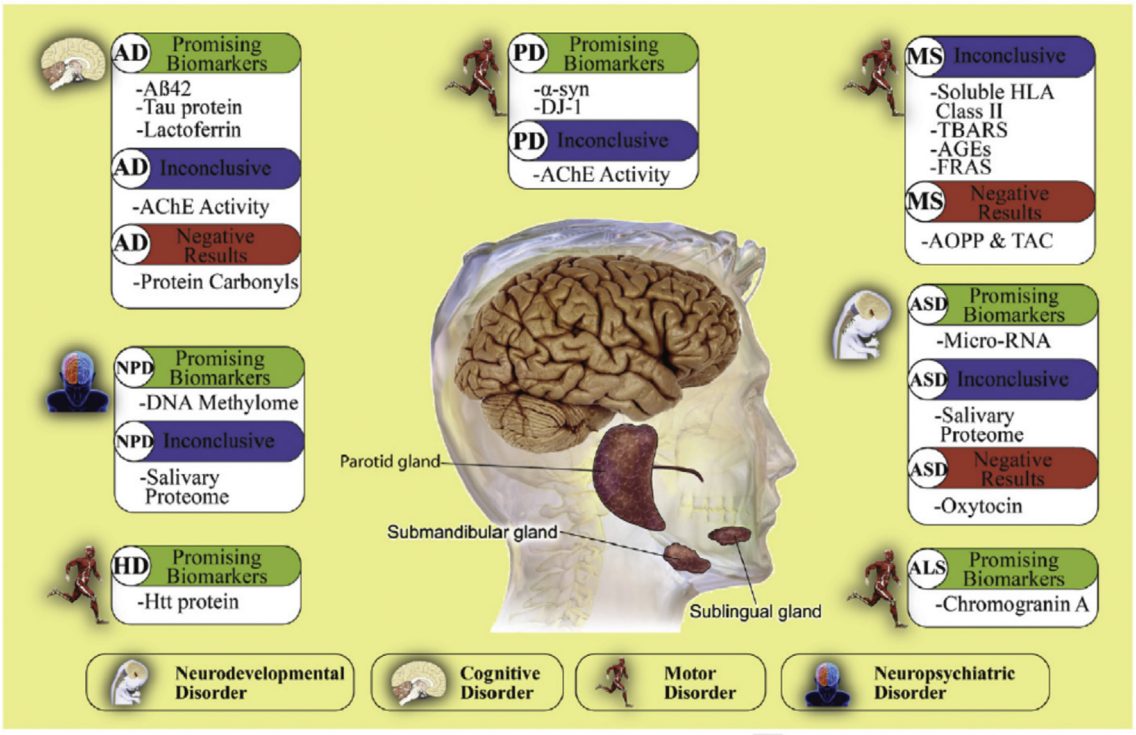

Fig. 1 The value of salivary biomarkers in neurological disorders. Biomarkers that may be useful for the diagnosis or prognosis of various neurological disorders are shown in green. Abbreviations used: AD : Alzheimer's disease; NPD : neuropsychiatric disorders; HD : Huntington's disease; PD : Parkinson's disease; MS : multiple sclerosis; ASD : autism spectrum disorder, ALS : amyotrophic lateral sclerosis. Figure kindly provided by Farah et al. [1]. See main article for more details on the various biomarkers.

disease (PD). When examining the concentration of α-syn in saliva, one study found that the proportion of the toxic oligomeric form of the protein was higher in patients with PD than in healthy controls [6]. Overall, levels of total α-syn in saliva are lower in patients with PD than in healthy controls, probably because of oligomerisation of free monomeric α-syn in the saliva of patients with PD, which reduces its total concentration [7,8]. Moreover, total levels of α-syn appear to correlate with disease severity in patients with PD, indicating its value not only as a diagnostic marker but also a prognostic one [6]. Similar to what is seen in PD, both huntingtin protein and amyloid beta peptides, the proteins implicated in Huntington's disease and Alzheimer's disease respectively, are capable of being detected in saliva and may be useful for diagnosis [9,10].

It is not just proteins however that offer insight into human health. Given that most neurological diseases are caused by a combination of genetic and environmental factors, much attention has turned to epigenetics – the array of mechanisms that regulate gene expression levels. Once such epigenetic mechanism is microRNA (miRNA), which are short non-coding RNA that interfere with gene expression by targeting complementary messenger RNA. In individuals with autism spectrum disorder (ASD), differences in the abundance of certain miRNA, compared to healthy controls, can be detected in post-mortem brain tissue [11]. Remarkably, such differences are also apparent in cells/tissues outside the brain, including olfactory mucosal stem cells [12] and saliva samples [13]. The target genes for the identified miRNAs were linked to neurodevelopment or had been previously associated with ASD. Clearly, there is a need to replicate these results, but they nonetheless offer the possibility of accelerating the detection of ASD, a spectrum of neurodevelopmental disorders that normally cannot be diagnosed before two years of age.

What works in the lab still has several barriers to face before it can be implemented in the clinic. A good diagnostic test must have high sensitivity and specificity, but also be high throughput, low cost and portable. The application of salivary diagnostics has seen rapid growth in recent years, with several emerging point-of-care-platforms allowing the analysis of various biomarkers (reviewed in Ref. [14]). With these developments, the key to early diagnosis of various neurological diseases could quite literally be on the tip of your tongue.

Spotlight on original articles

Epitope prediction: towards a Chlamydia *vaccine*

Chlamydia trachomatis is the world's most common sexually transmitted infection. Despite decades of research, there is still no vaccine against *C. trachomatis* approved for use in humans. In this issue of the *Biomedical Journal*, Russi et al. [15] hunt for B and T cell epitopes in a conserved pathogenic

protein of the bacterium, and report findings that may aid future vaccine development.

A staggering 131 million new cases of sexually transmitted infection are caused by *C. trachomatis* every year [16]. This pesky intracellular bacterium is a major cause of infertility and causes complications in both men and women [17]. Although the infection can be cured with antibiotics, most cases are asymptomatic leaving the bacterium free to wreak havoc with the reproductive system undetected. One major challenge to the development of a vaccine is that immune responses to naturally occurring *C. trachomatis* cause collateral damage to tissue [18]. Thus, identifying antigen candidates that bring about protection while avoiding harmful immune responses may overcome these safety concerns.

The Polymorphic Membrane Protein family (Pmp A-I) are promising vaccine candidates for *C. trachomatis* because they are immunodominant and located on the outer membrane of the bacterium [19]. These proteins function as autotransporters involved in the delivery of virulence factors [20]. PmpD in particular is an interesting candidate because it is highly conserved, showing 99.1% amino acid identity among *C. trachomatis* serovars [21]. However, the protein has a high molecular weight, which has hampered the solving of its 3D structure and identification of potential epitopes. To overcome these challenges, Russi et al. [15] turn to *in silico* modeling to predict B and T cell epitopes within PmpD.

First, Russi and colleagues used the online homology modeling tool Raptor X to predict the 3D structure of PmpD and verified the resulting modeling using programs for structural analysis. They then applied various programs to predict linear B cell epitopes along with discontinuous (i.e. conformational) B cell epitopes and T cell epitopes. This analysis revealed that potential B and T cell epitopes were distributed throughout the protein and identified many epitopes with strong affinity for MHC class I or MHC class II molecules. Ideally, epitopes that are followed up for vaccine development should elicit both a humoral and cellular immune responses. Indeed, during infection, neutralizing antibodies are produced [20] which probably help to control initial bacteria load. Once cells become infected with *C. trachomatis* however, they must be sought out and destroyed via cell-mediated immune responses. Fortunately, Russi et al.'s analysis identified six regions containing both B and T cell epitopes, which could form the basis for a vaccine that would activate T cells and the production of cytokines and antibodies.

Overall, these results will enable the rational selection of epitopes for screening and further development, which may bring the field one step further in the quest to develop a *C. trachomatis* vaccine.

Also in this issue

Reviews

Food safety in the 21st century
Fung et al. [22] discuss food safety in the 21st century, with a focus on state-of-the-art techniques to detect and prevent microbial contamination and the responsibility of today's governments to safeguard the world's food supply.

Fructose and metabolic syndrome: a lifelong story
High fructose consumption has been linked to metabolic syndrome (MetS), a complicated set of disorders with increasing prevalence [23]. Here, Lee et al. [24] review the effects of maternal fructose consumption on the development of MetS, and describe how exposure in utero or early life may bring about transcriptomic reprogramming that leaves individuals vulnerable to developing MetS in later life.

A glycoprotein with a role in insulin resistance
Huang et al. [25] summarize the emerging role of pigment epithelium-derived factor in lipid metabolic disorders. Although this secreted glycoprotein plays an important role in the hepatic lipid homeostasis, its increased expression in adipose tissue promotes the ectopic accumulation of fatty acids, tipping the balance in favor of insulin resistance and type 2 diabetes.

Original articles

Modelling a cancer testis antigen
XAGE-1 is a cancer/testis (CT) like antigen with four isoforms. In particular, the XAGE-1b variant is strongly overexpressed in lung adenocarcinoma [26] but the 3D structure of the protein remains elusive. Using a homology modeling approach, Tarek et al. [27] generate structural models of XAGE-1b from which they determined potential B and T cell epitopes. The expression of XAGE-1b is normally restricted to germ line cells, making XAGE-1b an ideal target for immunotherapy or vaccine against lung adenocarcinoma. The results reported here could inform such approaches.

No benefit of induction chemotherapy in head and neck cancer
Various tactics have been tried to improve outcomes in patients with advanced head and neck cancer. One such tactic is induction chemotherapy, which involves hitting the cancer hard with a high dose of anticancer drugs with the idea of making resulting radiotherapy more effective. The approach is commonly used in clinical practice, but several studies have questioned its value (reviewed in Ref. [28]). Huang et al. [29] add to the debate in a trial of patients with advanced pharyngeal and laryngeal squamous cell carcinoma, whereby patients were randomized to receive either induction chemotherapy before concurrent chemoradiotherapy or concurrent chemoradiotherapy alone. Their study reveals no benefit of induction chemotherapy for overall survival rate, arguing against its inclusion in standard care pathways.

Conflict of interests

The author declares no conflict of interests.

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