Ultrasensitive detection and glycan analysis of a prostate cancer biomarker

In recent years we have seen an increased frequency of diagnosed prostate cancer (PCa) cases accompanied with an enhanced mortality worldwide. Symptoms of an early-stage PCa can be mild or even absent, thus there is an urgent need to develop new, sensitive and reliable diagnostic test for identification of aggressive PCa forms. Nowadays, a level of prostate specific antigen (PSA) in blood/serum represents a gold standard for PCa diagnostics and monitoring of disease progression. The level of PSA is low in blood (less than 4 ng/mL) of healthy individuals and PCa patients usually have PSA level above 10 ng/mL. There is, however, a grey zone (4-10 ng/mL), when it is difficult for the doctor to decide if any treatment or operation is needed. This can result in unwanted treatment or operation even in cases when watchful waiting would be sufficient, seriously affecting patient´s quality of life. Therefore, an increased attention and effort has been devoted to the discovery of new ways for identification of early symptoms of the disease.

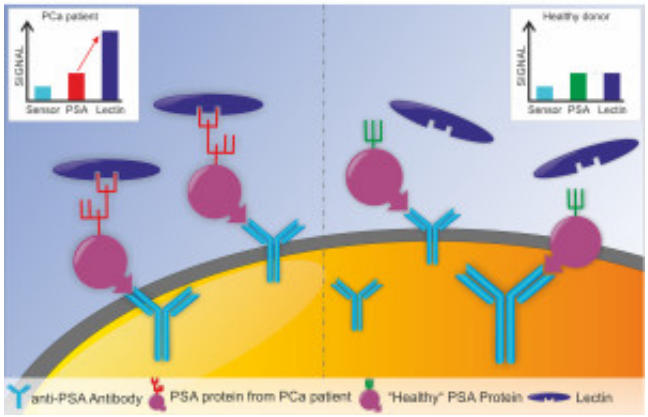

Fig. 1. A scheme showing detection of PSA level and glycan identity by the lectin either on PSA from healthy donor (no or weak interaction with lectin) or PCa patient (strong interaction with lectin).

Due to involvement of glycans (complex carbohydrates, see Ultrasensitive detection of influenza viruses by glycan biosensors) in various cellular processes, including cancer, changes in the glycan composition related to cancer could be used for this purpose. Glycans create a “sweet” coat on many proteins, including PSA. This is why changes in the glycan composition on PSA related to PCa have potential to provide additional useful information especially in cases, when PSA level in blood falls within a grey zone to help doctors to make a right decision.

Herein we propose a new electrochemical protocol of analysis, which could be applied for both measuring of PSA concentration and determination of PSA glycan composition on the same

biosensor surface, what has not been done yet (see Fig. 1). Our biosensor is based on an antibody attached on the biosensor surface selectively binding only PSA protein. When the PSA is bound to the biosensor, its level in the sample could be quantified. In the next step, the biosensor is incubated with lectins (glycan recognising proteins) to identify what particular glycan is present on captured PSA.

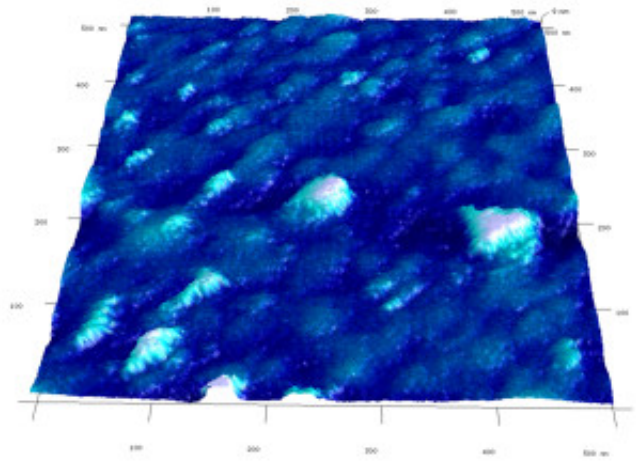

Fig. 2. Atomic force microscopy image of immobilised antibodies on the biosensor surface.

Thus, analysis of PSA and glycan identity present on PSA could be performed on the same surface using PSA level indicating PCa (i.e. 10 ng/mL). This electrochemical method of PSA detection and PSA´s glycan analysis was so sensitive that 100 million time diluted PSA level could be analysed, as well. Such extreme sensitivity of detection of PSA was possible only due to an optimised design of the biosensor configuration and assay conditions. Novel characterisation techniques such as atomic force microscopy (AFM) helped us to understand, which density of antibody on the biosensor surface is optimal to built-up an ultrasensitive biosensor device (Fig. 2). The future diagnostic potential of the biosensor with nano-scale patterning protocol, low sample consumption and low cost fabrication has to be still shown.

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