

## Editorial

# Biomarkers as a Part of Serum Protein Interactome

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Serum protein biomarkers form complexes with other serum proteins. The studies of these interactions are important for discovering new biomarkers which are used for disease diagnostics and to follow results of disease treatment.

During the past decade intensive studies were undertaken to discover specific proteins, biomarkers, which could be used in clinical practice to diagnose disease and to follow the progress of medical treatment [1,2]. In most of these studies human blood serum was used as the predominant source for biomarker discovery. This very complex biofluid contains many thousands of proteins representing nearly all the protein families present in the body as blood vessels come into contact with all organs and tissues. The most abundant proteins – albumin, immunoglobulins, transferrin and several others – comprise more than 90% of the serum proteome. Intact cell proteins and their fragments are present but only in low abundance and identifying specific biomarkers within these minor fractions is of particular value. Therefore, the methods used to discover biomarkers must not only be specific but very sensitive as well.

Despite tremendous efforts, only a few validated protein biomarkers are currently in use as diagnostic indicators. These include prostate specific antigen (PSA), cardiac protein troponin, and epithelial glycoprotein CA125 among others. Difficulties in identifying new biomarkers of clinical value have been attributed to several factors. Firstly, blood serum is a very complex proteome system: prior to using modern techniques such as mass spectrometry (MS), preliminary fractionation is necessary, in order to deplete highly abundant proteins from the serum sample. These preliminary steps must be standardized to avoid adding variability to the results obtained [3]. In addition they take a significantly long time. Another important problem is linked to the impact of individual variations on serum samples; factors as age and gender also play a part. Proteolytic and other type of modifications of serum proteins also must be taken into account. Furthermore, validation of the biomarkers discovered is a time-consuming and costly process.

Currently, two powerful methods are used to discover biomarkers – mass spectrometry [4-7] and immunological approaches [8,9]. Both are specific and sensitive. To find a protein which could constitute a marker for a pathological condition, dozens of serum samples must be examined. Significantly more samples must be studied to verify if the protein is a true marker of a given disease and can be used in clinical diagnostics and

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trials [10]. However, an increase in sample numbers results in the parallel rise of the cost of the analysis and in the time taken to perform it. All these factors hinder the discovery of new biomarkers.

Another critical factor that could adversely affect biomarker identification concerns interactions between protein molecules. The interactions are one of the most important properties of proteins. It was estimated that the number of protein-protein interactions (interactome) in humans estimated to be very large – about 650,000, however, less than 1% of them has been identified [11]. High protein concentration in serum (6-8%) facilitates such interactions. The considerable reactivity of abundant serum proteins is well-known. Immunoglobulin G molecules are able to form complexes with protein molecules of animal, bacterial and viral origin [12]. Various non-immune IgG complexes – IgG-anaphylatoxins, IgG-prolactin, IgG-heat shock protein Grp94 have been found in human serum [13]. Two factors facilitate high ability to form complexes with IgG – firstly, the special structure of C1 domains which comprise constant parts of IgG peptide chains, and the secondly, exceptional flexibility of Ig molecules [14] which enable the formation optimal configurations for various binding interactions. The major abundant serum protein – albumin (which constitute 50% of all serum proteins), has long been known as a carrier for proteins, as well as for hormones and other important substances.

The presence in the circulation of complexes of biomarkers with abundant proteins has been shown experimentally [15]. Affinity-based method under mild conditions was carried out in this study to isolate the six most abundant proteins – albumin, IgG, IgM, IgA, transferrin and apolipoprotein. About 200 proteins associated with these abundant proteins were then isolated. Among the separated proteins twelve (6%) were biomarkers which are currently used in clinical practice, among them prostate specific antigen, pregnancy plasma protein A, meningioma-expressed antigen and dehydropteridine reductase. Furthermore, other proteins of clinical interest were found, such as morphogenetic protein 3b, prostate transglutaminase, paraneoplastic antigen MA1, glucosylasparaginase, coagulation factor VII precursor, and ryanodine receptor 2.

The association of proteins with highly abundant serum proteins is important for several reasons. First of all, such

interactions extend the persistence of proteins in the circulation for longer period of time, especially if they have a molecular mass below that of the kidney filtration cutoff (~45 kDa). In forming complexes with larger, long-lived, molecules small proteins and peptides escape rapid clearance as well as degradation by proteolytic enzymes thereby increasing protein half-life in the circulation. For proteomic studies of serum proteins in low abundance the fraction of most abundant proteins is usually removed. However, following such procedure various protein molecules including clinically useful biomarkers associated with albumins, immunoglobulins and other abundant proteins may also be removed, and thus escape detection. On the other hand, the formation of serum protein complexes results in the enrichment of proteins present in the circulation in low concentrations, among them proteins with clinical importance.

Serum contains thousands types of proteins synthesized by liver, lymphoid and other cells and a large numbers of secreted and shed protein molecules as well as proteins released during tissue cell disintegration [16]. Traces of diverse non-self proteins (e.g. bacterial, fungi and viral products) could also be present in blood. There is little doubt that these molecules form a large network (interactome), due to multiple protein-protein interactions. The binding character of these interactions is not well studied as well as their specificity. However, based on the above, it is obvious that biomarkers, clinically important proteins, could interact with the highly abundant serum proteins and participate in the serum interactome.

The study of all these complexes is only in its beginning. Several aspects of the interactions must be clarified. Some are related to structural problems, such as the spatial organization of proteins in a complex, and the structure and localization of the protein interaction sites. Others involve functional aspects such as what the type of interactions (electrostatic, hydrophobic etc) and how strong they are. Without question, proteins in low abundance also form networks. A deeper understanding of such networks, in which validated biomarkers are present, is important for the more effective biomarkers discovery [17].

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