PERSPECTIVES ON THE DEVELOPMENT OF NEW PEPTIDE DISEASE BIOMARKERS WITH BIOMEDICAL APPLICATIONS

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Abstract: *The present paper aimed at reviewing the existing studies regarding the active peptides involved in normal biological and diseases processes, which could become candidate biomarkers for diseases. Detection of disease-associated proteins and peptides is mainly based on proteomics and peptidomics technologies. Here, we describe today's available research on protein and peptide markers for several diseases and the progress accomplished in applying the "-omics" approaches for the discovery of new effective biomarkers.*

Keywords: *peptides, biomarkers, proteomics, peptidomics*

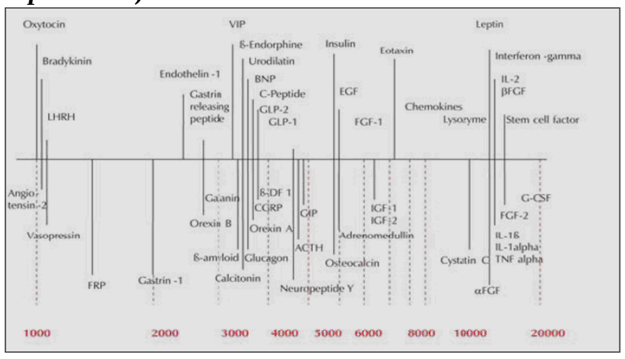
Rezumat: *Articolul prezent îşi propune să prezinte cercetările actuale privind peptidele biologic active care sunt implicate în procesele biologice normale şi patologice şi care pot deveni astfel candidaţi eficienţi pentru biomarkeri specifici anumitor boli. Identificarea peptidelor şi proteinelor asociate bolilor se bazează actual pe tehnologiile proteomice şi peptidomice. Se descriu datele existente în literatura de specialitate privind markerii biochimici bazaţi pe proteine şi peptide, precum şi progresele realizate în aplicarea tehnologiilor "-omice" pentru descoperirea de noi biomarkeri eficienţi.*

Cuvinte cheie: *peptide, biomarkeri, proteom, peptidom*

INTRODUCTION

The study of peptides represents a hot topic of biochemical research, as these compounds play a key role in many processes with high impact on human health. Important peptides for biological processes are shown in picture no. 1.

Picture no. 1. Main bioactive peptides from human body fluids and tissues. Molecules are presented in the order of their molecular weight (by *DigiLab, Peptidomics*)



In the near future peptides will also reclaim an important place as potential biomarkers of certain diseases. At present, biochemical and cellular parameters are measured by clinical chemists to assess disease development and therapy. Peptides/proteins are released in certain body fluids and tissues under specific biological conditions. Detection and identification of such specific biomolecules are important steps in discovery and validation of novel biomarkers and in the design of specific tests. The current NIH-FDA accepted definition of a biomarker refers to an indicator of normal biologic/pathogenic processes or pharmacological response to a therapeutic intervention [1].

Literature data report several analytical techniques to measure the level of a biomarker in body fluids (plasma, serum, urine, faeces) or tissues. Serum is the most accessible material used for biomarker analysis. However under certain conditions of blood sampling, proteases can modify the concentration of peptide products leading to misinterpretation of obtained data. Considering these false results, *The Human Proteome Organisation* (HUPO) focused on establishing standard operating procedures for blood collection, storage and processing, in order to detect only the valid peptide and protein disease biomarkers.

Biomarker research has shown that different peptides are involved in diabetes, breast cancer, blood pressure regulation, multiple sclerosis, obesity, Alzheimer's disease, excessive alcohol consumption or immunological diseases [2-6]. Some validated peptide-based diagnostic and biomarker products are known, *e.g.* C-peptide, proβ-type natriuretic peptide, β-amyloid-peptides. Validation of new biomarkers is a very long and complex process requiring the study of large populations over a great period of time. An efficient biochemical diagnostic marker should allow early detection of disease and produce low false positive and negative results.

PEPTIDE-BASED BIOMARKERS IN MEDICINE

In the present review, some important peptide and protein biomarkers for cancer, cardiovascular and neurological diseases are described.

Traditional *cancer diagnosis* is based on clinical and histopathological data which could become insufficient in case of atypical or subtype tumors. For this

reason oncology research is directed to the development of effective biomarkers not only for earlier tumor diagnosis or therapy decision but also for identification of high-risk individuals. Several biomarkers are used for different types of tumors. Specific biochemical markers for colorectal cancer (CRC) are carcinoembryonic antigen (CEA), CA 19-9, CA 242, tissue polypeptide antigen (TPA) and tissue inhibitor of metalloproteinases-1 (TIMP-1) [7]. Recently, new serum and tumor tissue peptide biomarkers - human neutrophil peptides (HNP1-3) and human α-defensin 6 (HD 6) were detected in CRC patients [8-9]. Unfortunately the latter cannot be used for early and highly specific detection of CRC. Ovarian cancer is one of the most aggressive gynecological malignancies causing high mortality. Currently research is focused on development of new specific and sensitive serum biomarkers for early detection of ovarian tumors. Several glycoprotein biomarkers were described, *e.g.* CA-125, CA 15- 3, CA 72-4, CA 19-9 [10-11]. These biological markers are used separately or in combination. Increased levels of haptoglobin α-subunit were also detected in sera of ovarian cancer patients setting this protein as a possible diagnosis biomarker [12]. Regarding pancreatic cancer, only few biomarkers are known. One biomarker candidate is NAD(P)H: quinone oxidoreducatse 1 (NQOR 1) (or DT-diaphorase) which was detected in pancreatic tumor tissue but also in other human tumors [13]. It was also highly associated with smoking habit. Another type of cancer which need a continuous research for discovery of effective biomarkers is breast cancer, as to date only few markers have gained prognostic value. The currently used biological markers are estrogen receptor (ER), progesterone receptor (PR) and HER-2 [14]. Regarding the hepatocellular carcinoma (HCC), the neutrophil-activating peptide 2 (NAP-2) was identified as a specific peptide biomarker candidate.

Cardiovascular disease continues to represent a major problem worldwide causing high morbidity and mortality. It was reported a good relationship between immune system, inflammatory processes and the progression of atherosclerotic disease and rupture of plaque [15]. Currently used cardiac biomarkers are creatine kinase and it's MB-isoenzyme, troponin, C-reactive protein (CRP), myeloperoxidase, brain or B-type natriuretic peptide (BNP) and N-terminal pro-hormone brain natriuretic peptide (NT-proBNP). Specific biomarkers candidate of cardiac health are natriuretic peptides, ANP and BNP, which are secreted by the heart in proportion to cardiac transmural pressures [16]. Despite of the current biochemical markers there is a great demand for new cardiac biomarkers from proteomics/metabolomics and a multimarker approach.

Regarding *neurological diseases*, Alzeihemer's disease -the most common form of dementia characterized by degeneration of neurons and their synapses- was intensively studied. Development of novel biomarkers for its early diagnosis will lead to efficient treatment strategies.

The current biochemical marker of Alzeihemer's disease is the amyloid β-peptide (Aβ) from cerebrospinal fluid [17]. Later, changes in the level of shorter (Aβ40) and longer hydrophobic (Aβ42) forms of Aβ proteins were found responsible for neuronal damages [18]. It was shown that the β-sheet secondary structure of amyloid peptides is responsible of their neurotoxicity. Aβ40 predominates in vascular amyloid and cerebrospinal fluid, while Aβ42 which is more hydrophobic aggregates in senile plaques and consequently diminishes its level in cerebrospinal fluid. Aβ42 is positive in cerebrospinal fluid very early in the disease process, before clinical dementia. Several studies have shown that these biomarkers are specific also for other forms of dementia or other neurodegenerative disorders (Creutzfeldt–Jakob disease, frontotemporal dementia, vascular dementia, Lewy body dementia). The recommendation is to use the cerebrospinal fluid Aβ42 biomarker together with clinical examination, brain-imaging techniques and other cerebrospinal fluid biochemical markers [19].

PROTEOMICS AND PEPTIDOMICS TECHNOLOGIES FOR BIOMARKER DISCOVERY

One of the most important technology for biomarkers discovery rely on proteomics and more recently peptidomics approach as it is known that usually, in living systems peptides are released from protein precursors by the action of proteases. Consequently, the level of these biomolecules changes dynamically reflecting normal biological processes, disease processes or response to specific therapeutic treatment. These revolutionary technologies will provide new tools in disease diagnostic, prognostics and therapeutics by analyzing complex mixtures of proteins and peptides.

Proteomics deal with the analysis of all proteins encoded by the genome and their post-translational modifications. Proteomics approach was investigated for different diseases *e.g.* infectious diseases, auto-immune diseases, vascular disease, breast, prostate and liver cancer [20-24]. A much reduced number of research articles deals with the peptide analysis of an organism-named *peptidomics* in analogy to proteomics [25]. Peptide profiling process starts with the peptide extraction from tissue, blood or urine, follows with the chromatographic separation, detection and identification of each fraction by mass spectrometry, differential peptide display (comparative analysis of data for disease and control groups) and sequence identification. Peptide samples which require few starting material and little manipulation of it, are considered the most suited molecules for analysis by different mass spectrometric techniques: matrix-assisted laser desorption/ionization MS (MALDI-MS), electrospray ionization MS (ESI-MS), or combined techniques such as matrix-assisted laser desorption/ionization combined with time-of-flight MS (MALDI-TOF-MS) or TOF-TOF-MS.

CONCLUDING REMARKS

Peptides are promising biomolecules useful as indicators for diagnosis of diseases or for monitoring response to therapeutic intervention. In the present review, protein/peptide biomarkers for several specific diseases have been outlined.

The new technologies of proteomics and peptidomics give the opportunity to discover new qualified biomarkers for diseases by analyzing the proteome and peptidome of any tissue and the expression of the entire proteome/peptidome in different physiological conditions. Despite of these advanced techniques which revolutionized medicine, future work is needed to have complete detection of specific diseases biomarkers and to experiment the efficiency of different biomarkers combinations. Analytical validation of biomarkers represents a major challenge for the scientific community being a difficult and time consuming process.

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