Advanced Drug Delivery Reviews 64 (2012) 1522–1531

Contents lists available at SciVerse ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Nanoproteomics enabling personalized nanomedicine ☆

Claudio Nicolini [a,b,c,*], Nicola Bragazzi [a,d], Eugenia Pechkova [a,b]

[a] Nanobiotechnology and Biophysics Laboratories, Department Experimental Medicine, University of Genoa, Italy
[b] Nanoworld Institute Fondazione EL.B.A Nicolini Bergamo, Italy
[c] Biodesign Institute, Arizona State University, Tempe, USA
[d] School of Public Health, Department of Health Sciences (DISSAL), University of Genoa, Italy

ARTICLE INFO

Article history:
Received 8 April 2012
Accepted 28 June 2012
Available online 20 July 2012

Keywords:
QMC_F
QMC_D
MS
APA
Leader Genes
NAPPA

ABSTRACT

Nucleic Acid Programmable Protein Arrays utilize a complex mammalian cell free expression system to produce proteins in situ. In alternative to fluorescent-labeled approaches a new label free method, emerging from the combined utilization of three independent and complementary nanotechnological approaches, appears capable to analyze protein function and protein–protein interaction in studies promising for personalized medicine. Quartz Micro Circuit nanogravimetry, based on frequency and dissipation factor, mass spectrometry and anodic porous alumina overcomes indeed the limits of correlated fluorescence detection plagued by the background still present after extensive washes. This could be further optimized by a homogeneous and well defined bacterial cell free expression system capable to realize the ambitious objective to quantify the regulatory protein networks in humans. Implications for personalized medicine of the above label free protein array using different test genes proteins are reported.

Contents

1. Introduction

Personalized nanomedicine is a promising and formidable tool for targeted and more rationale therapeutics that really fits individual patient's needs emerging from the combination of personalized medicine defined as "as the prescription of specific treatments best suited for an individual taking into consideration both genetic and other factors that influence the response to therapy" [1–3] and of nanomedicine also defined as "the interplay of Bioinformatics and Nanotechnology to a previously unforeseeable level" [4,5].

Integrated genomics–proteomics data are useful for finding disease biomarkers [6–8] and for providing a more precise diagnosis and prognosis in the frame of translational medicine: they can give an added value to the clinical assessment of the patient, as well as they can even predict the risk of a not yet clinically observable disease. A very recent and complex publication [9] has shown that iPOP (integrative personal Omics profile) resulting from the combination of different Omics-based data can be a promising approach to manage proactively individual health: dynamic information of how genes interact with environment and change their expression throughout the time could be extracted and correlated with clinical implications. In the specific case type-2 diabetes was predicted much earlier than its clinical onset: thus medicine can become quantitative, predictive and preventative [10,11].

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Personalized nanomedicine".

* Corresponding author at: Biophysics University of Genova Medical School, Via Antonio Pastore 3, 16132, Genova, Italy. Tel.: +39 010 353 38217; fax: +39 010 353 38215.
E-mail address: cnicolini@ibf.unige.it (C. Nicolini).

0169-409X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2012.06.015

Diseases such as cancer ([12] and references therein, [13,14]), obesity and diabetes [15], transplanted organ rejection [16] and hypertension [17] are complex biological phenomena, being sometimes highly heterogeneous among patients despite similar clinical features. In the last decades, patients can be affected by many chronic diseases [18,19] throwing classical therapeutic approaches into crisis. "Classical medicine" fails to achieve its goal to cure individuals, and also molecular approach-based medicine risks to be unsuccessful if it does not take into account individual variations of the same disease [20]. A network approach for molecular medicine is emerging [21–28] in order to find precisely the target for an optimized drug therapy [29]. An alternative strategy for cancer biomarker validation and cancer treatment based on the decoding of the serum proteins patterns is indeed emerging [30] and the technical advancement in measurements and the information derived from system biology should permit to develop tailored treatment of personalized medicine in the future.

Moreover, new developments in proteomics tools emerging from the combination of both microfluidic chips (like the microfluidic integrated approach for identifying protein isoforms, in [31]) and an ad hoc designed protein-capture agents (like the SOMAmers in the SOMAscan technology, in [32]) can make the dream of a comprehensive proteomics become true.

In Fig. 1 we introduce a protocol starting from the collection of patients' samples (saliva, blood, urine or others) and from the analysis of the differentially expressed genes using both statistical and not-statistical analyses with Leader Genes [33] and DNASER [34] approaches, introduced time ago as tools for molecular genomics enabling the selection of few important highly interconnected genes. These ab initio targeted microarray-based bioinformatics and nanogenomics [35] lead to few identified genes that can be expressed in a cell-free environment thanks to Nucleic Acid Programmable Protein Array (NAPPA) technologies [36,37] which utilizes a complex mammalian cell free expression system to produce proteins in situ. In alternative to fluorescently-labeled approaches [38] a wide range of nanotechnological approaches have been recently introduced to characterize NAPPA microarrays, all critically discussed [39]. Label-free NAPPA technology, in combination with drug nanodelivery [40] and protein nanobiocrystallography [41,42] and its possible ongoing developments using anodic porous alumina (APA) and bacterial cell-free expression system, appear to form a single approach, named nanoproteomics [43], capable of effectively solving the numerous problems still present in medical diagnosis and therapy. The emerging label free NAPPA method recently introduced [44] consists of the combined utilization of QMC_D nanogravimetry, mass spectrometry and APA (Fig. 1) as the last step of the protocol for personalized medicine (Fig. 1) being optimal in analyzing protein function and protein–protein interaction. Target protein microarrays provide a powerful tool for high-throughput (HT) analysis of protein–protein or small molecules–protein interactions. Common protein microarrays are generated by separately spotting purified protein on the arrays with linkage chemistries. However, due to the complicated work associated with HT protein production and purification, and the concerns regarding protein stability during storage before and after being spotted on the array, a significant improvement to the target protein microarrays is represented by NAPPA which allows for functional proteins to be synthesized in situ directly from printed cDNAs just in time for assay [36–39]. Printing cDNA, rather than proteins, eliminates the need to express and purify proteins separately and produces proteins "just-in-time" for the assay, abrogating concerns about protein stability during storage [45,46]. Label free nanotechnologies, previously used to study molecular interaction and adsorption of different biomolecules, are here applied to functionalized NAPPA surfaces [39–44] to enable the detection of proteins and their interactions without the need for labeling reagents with tags. The detection of low-abundant biomarkers from biological fluids (e.g., blood, urine or saliva) requires high throughput detection techniques and in this sense nanoproteomics provides the robust analytical platform for personalized medicine, as needed for real-time and label-free sensitive detection of low-abundant proteins [43,44]. In traditional labeling methods (Fig. 2) of both nanogenomics and nanoproteomics a fluorescent molecule is

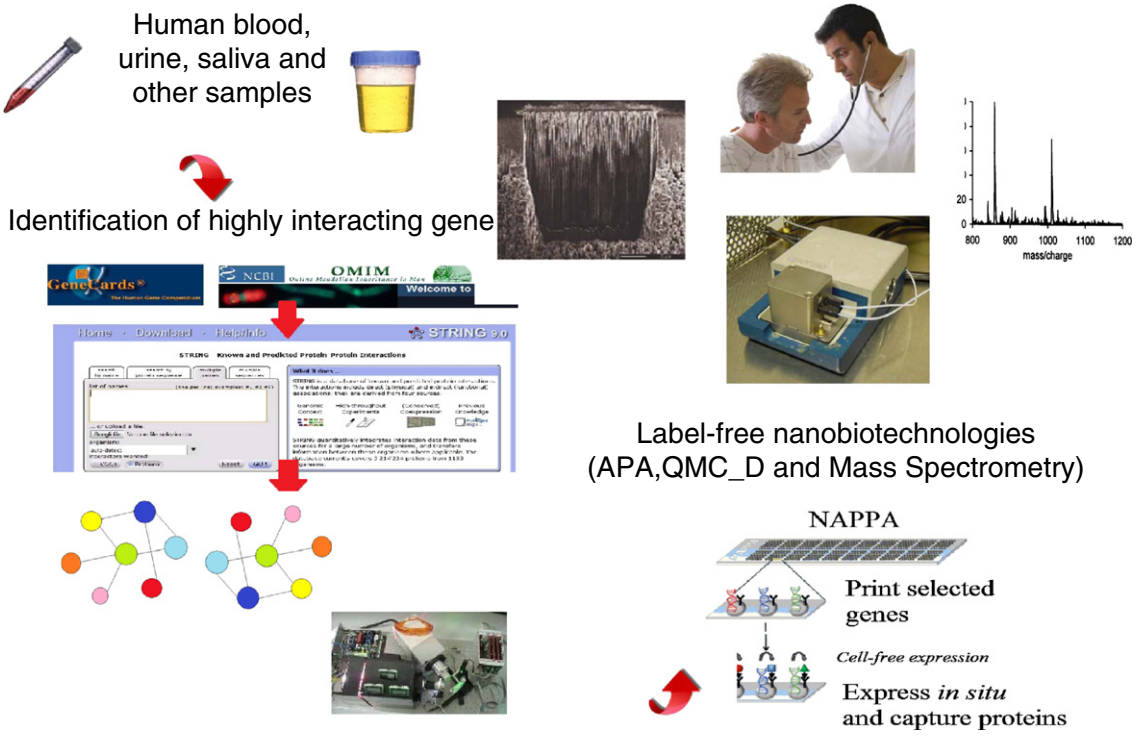

Fig. 1. Protocol of NAPPA-based nanoproteomics for personalized medicine. After collecting the samples from the patients, differentially expressed genes (DEG) are studied according to both statistical and not-statistical techniques and Leader Gene approach. Thus only few genes of interest are selected (genomic signature), they are expressed via NAPPA technology and protein–protein interactions (proteomic signature) are fully characterized via label-free nanobiotechnologies (namely nanogravimetry, Anodic Porous Allumina, mass spectrometry), presently overcoming the limitations of labeled technologies.