Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Comparative proteomics in alkaptonuria provides insights into inflammation and oxidative stress

Daniela Braconi[a], Giulia Bernardini[a], Alessandro Paffetti[a], Lia Millucci[a], Michela Geminiani[a], Marcella Laschi[a], Bruno Frediani[b], Barbara Marzocchi[a], Annalisa Santucci[a,*]

[a] Dipartimento di Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, 53100, Siena, Italy
[b] Dipartimento di Scienze Mediche, Chirurgiche e Neuroscienze, Università degli Studi di Siena, 53100, Siena, Italy

ARTICLE INFO

Article history:
Received 16 June 2016
Received in revised form 9 August 2016
Accepted 16 August 2016
Available online 31 August 2016

Keywords:
Advanced oxidation protein products
Biomarkers
Osteoarthritis
Plasma
Serum
Serum amyloid a

ABSTRACT

Alkaptonuria (AKU) is an ultra-rare inborn error of metabolism associated with a defective catabolism of phenylalanine and tyrosine leading to increased systemic levels of homogentisic acid (HGA). Excess HGA is partly excreted in the urine, partly accumulated within the body and deposited onto connective tissues under the form of an ochronotic pigment, leading to a range of clinical manifestations. No clear genotype/phenotype correlation was found in AKU, and today there is the urgent need to identify biomarkers able to monitor AKU progression and evaluate response to treatment. With this aim, we provided the first proteomic study on serum and plasma samples from alkaptonuric individuals showing pathological SAA, CRP and Advanced Oxidation Protein Products (AOPP) levels. Interesting similarities with proteomic studies on other rheumatic diseases were highlighted together with proteome alterations supporting the existence of oxidative stress and inflammation in AKU. Potential candidate biomarkers to assess disease severity, monitor disease progression and evaluate response to treatment were identified as well.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Alkaptonuria (AKU) is an ultra-rare inborn error of metabolism associated with a defective catabolic pathway of the aromatic amino acids phenylalanine and tyrosine (Phornphutkul et al., 2002). In affected individuals, mutations of the homogentisate 1,2-dioxygenase (*HGD*) gene cause the production of a defective HGD enzyme and inability to break down homogentisic acid (HGA) (Nemethova et al., 2015). Consequently, when compared to the healthy population, HGA levels in serum of alkaptonuric individuals are significantly raised (though varying greatly due to dietary intake of precursor aminoacids). Serum HGA is reported to range in AKU from 5.8 to 400 μmol/l (Angeles et al., 1989; Bory et al., 1990; Hughes et al., 2015; Ranganath et al., 2016) while in healthy individuals is almost null [0.014–0.0714 μmol/L, according to (Deutsch and Santhosh-Kumar, 1996)]. In AKU, excess HGA is partly excreted in the urine, partly accumulated within the body and deposited onto connective tissues as an ochronotic pigment, leading to a range of clinical manifestations. The exact composition and mechanisms of production of such a pigment are still obscure; however, AKU patients undergo a premature and disabling form of severe arthritis-like joint damage (ochronotic arthropathy) and often develop heart valve disease. Today, there is still no licensed treatment available for AKU.

Genetic analysis, though informative, failed so far in clarifying mechanisms of AKU and ochronotic arthropathy. No clear genotype/phenotype correlation has been determined in AKU, likely due to variability in residual HGD enzymatic activity and patients' lifestyle. A disease severity scale cannot be based on HGA systemic levels nor on other specific molecular parameters, and to this aim only questionnaire-based evaluations can be undertaken (Cox and Ranganath, 2011; Ranganath et al., 2016).

In the past years, several *in vitro* and *ex vivo* AKU models were developed and characterized, allowing a deeper understanding of AKU molecular mechanisms. Besides the well-known relevance of HGA-induced oxidative stress (Braconi et al., 2012, 2011, 2010a, 2010b, 2015, 2013; Millucci et al., 2014b; Tinti et al., 2010), novel insights were provided into the interaction between serum proteins and benzoquinone acetate, which is produced by spontaneous HGA oxidation (Braconi et al., 2011). AKU was also found to be associated to a secondary amyloid-A (AA) amyloidosis (Millucci et al., 2012), with amyloid deposits affecting different tissues and organs (Millucci et al., 2014a, 2014b, 2014c) and co-localizing with

* Corresponding author at: Università degli Studi di Siena – Dipartimento di Biotecnologie, Chimica e Farmacia, via A. Moro 2, 53100, Siena (SI), Italy.
E-mail address: annalisa.santucci@unisi.it (A. Santucci).

http://dx.doi.org/10.1016/j.biocel.2016.08.016
1357-2725/© 2016 Elsevier Ltd. All rights reserved.

ochronotic pigment (Braconi et al., 2015, 2013; Millucci et al., 2015a, 2014a, 2014b, 2014c, 2015b, 2012; Spreafico et al., 2013). Chronic inflammation has a central role in the induction of AA amyloidosis, where it is also considered triggering event for the generation of oxidative stress. Several signs of local inflammation and oxidative stress were reported in AKU cells and tissues after the very first observations of Martin and Batkoff that HGA, when injected locally in joints, could produce inflammatory reactions and ROS (Martin and Batkoff, 1987). Indeed, in the proximity of HGA-induced ochronotic pigment, lymphocytes and macrophages are often found (Millucci et al., 2014a, 2014b; Selvi et al., 2000; Taylor et al., 2010). Oxidized lipids are known to be pro-inflammatory, and lipid peroxidation (LPO) is a hallmark of amyloidosis. In AKU, an intimate connection was highlighted among ochronotic pigment, amyloidosis and LPO [(Millucci et al., 2014a, 2014b) since LPO positively correlated with ochronotic areas and SAA deposits (Millucci et al., 2014a, 2014b). Furthermore, AKU tissues were found to contain myelin figures, which are indicators of both LPO and membrane degeneration (Millucci et al., 2014a, 2014b). Local inflammation was also highlighted in AKU chondrocytic cells that, once cultured in vitro, were found to release nitric oxide and pro-inflammatory interleukins (Braconi et al., 2012). Since administering HGA in vitro to normal cells induces LPO, release of pro-inflammatory mediators (SAA included) and amyloid (Spreafico et al., 2013; Tinti et al., 2010, 2011a, 2011b), HGA was hypothesized to be the primary cause of local inflammation, oxidation and amyloidosis in AKU. A chronic inflammatory status was proven also systemically in AKU patients, who often show elevated blood levels of Serum Amyloid A (SAA) (Millucci et al., 2015a, 2014a, 2014b, 2014c, 2012) and pro-inflammatory cytokines (Millucci et al., 2014b; Spreafico et al., 2013), pointing out that AKU is an inflammatory amyloidogenic multisystemic disease, much more complex than thought so far. Further evidence of the HGA-induced oxidative stress was provided in several AKU models and cells. HGA-treated human serum revealed the presence of oxidatively modified proteins concomitantly with alteration of GSH-related enzymes and thiol depletion (Braconi et al., 2011), likely compromising plasma antioxidant capacity (Giustarini et al., 2012). HGA-induced protein oxidation was also shown in HGA-treated chondrocytes (Braconi et al., 2010b) and AKU chondrocytes (Braconi et al., 2012). Once oxidized, proteins are more prone to aggregation and loss of functions, possibly promoting in AKU the production of the ochronotic pigment and leading to an impaired ability to cope with oxidative insults (Braconi et al., 2015, 2013). Thus, it seems likely that, besides inflammation, AKU patients experience also significant oxidative stress due to the high systemic levels of HGA and its by-products.

Despite these recent findings, which helped the clarification of AKU mechanisms, today there is still the urgent need to identify biomarkers able to monitor AKU progression and evaluate response to treatment. For the moment, both might be assessed only through questionnaire-based interviews to patients. In order to fill this gap, we undertook the present work to provide the first proteomic characterization of plasma and serum samples of six alkaptonuric individuals. Our work allowed us to highlight proteomic alterations in AKU samples showing interesting similarities with other rheumatic diseases and further supporting the existence of HGA-related oxidative stress and inflammation in AKU.

2. Materials and methods

2.1. Subjects and samples

The investigated subjects were selected from a cohort of Italian alkaptonuric patients who referred to the Rheumatology Unit of Siena University Hospital (Prof. M. Galeazzi) for ochronotic complications of AKU. These patients underwent routine hematological analysis and all of them, after informed consent and in accordance with the Declaration of Helsinki, agreed to donate their blood samples (serum or plasma) for this study. Samples were collected in fasting conditions and kept frozen at $-80\,^{\circ}C$ until analysis. For 2D-PAGE analysis, a commercially available pooled healthy human serum was used as a control (Bio-Rad, C1000006).

The study received approval from the Local Ethics Committee. Demographics and clinically relevant information on patients selected for the study are schematically reported in Table 1.

2.2. SAA, CRP and AOPP

SAA levels were assessed by means of a commercial ELISA assay (Invitrogen-Life Technologies) as described previously (Millucci et al., 2015a). Advanced oxidation protein products (AOPP) were measured using the microplate assay by Witko-Sarsat et al. (Witko-Sarsat et al., 1996). C-reactive protein (CRP) levels were measured by immunoturbidimetry (Cobas C 501, Roche/Hitachi).

2.3. 2D-PAGE and image analysis

Protein content of each sample was determined through Bradford's assay. 100 μg of proteins/sample were submitted to 2D-PAGE and silver ammoniacal staining as previously described (Braconi et al., 2012, 2010b). Digitalized images were obtained with ImageScanner III and analyzed by ImageMaster software (GE Healthcare BioSciences). Due to the different type of AKU samples that were available to us (plasma and serum), all of the spots identified as "fibrinogen" in the gels obtained from AKU plasma were manually selected and omitted from the qualitative and quantitative comparative analyses. Then, the increasing/decreasing index (fold-change) was calculated as the ratio of spot% relative volume between the different gel maps; for multiple spots identified as different molecular species of a same protein, the total% relative volume was calculated and taken into account. Protein spot identification was carried out by gel matching with the master gel of human plasma retrieved by http://world-2dpage.expasy.org/swiss-2dpage

2.4. Statistical analysis

The experiments were carried out in triplicate; data are presented as mean values with standard deviation. Statistically significant thresholds of 2.0 and 0.5 for fold-change values in protein relative abundance ratios calculated as AKU/control were set to highlight overexpressed and underexpressed proteins, respectively.

3. Results and discussion

In this study, we investigated the plasma/serum proteome profiles of AKU subjects in comparison to healthy controls. Six AKU subjects (one female and five males, range 39–66 years) who referred to the rheumatologic clinic of Siena University Hospital for ochronotic complications of the disease were selected. All of them had previously underwent joint replacement surgery and complained about articular disorders, arthropathy and joint pain together with other co-morbidities (Table 1). All of them presented SAA pathological levels above the reference value; CRP levels were slightly increased only two subjects (AKU#2 and 3), and AOPP levels were above the reference threshold in 3 out of 4 tested cases (AKU#1, 2 and 3) (Table 2).

For the comparative proteomics, a classical gel-based 2D-PAGE approach was used with direct processing of the samples without removal of the most abundant proteins. We are aware that depletion of multiple high-abundance blood proteins improves protein

Download English Version:

https://daneshyari.com/en/article/5511475

Download Persian Version:

https://daneshyari.com/article/5511475

Daneshyari.com