JOURNAL OF PROTEOMICS 113 (2015) 194–205

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Urine, peritoneal fluid and omental fat proteomes of reproductive age women: Endometriosis-related changes and associations with endocrine disrupting chemicals

Katherine E. Williams[a,b,c], Olga Miroshnychenko[a], Eric B. Johansen[a], Richard K. Niles[a], Rajeshwari Sundaram[d], Kurunthachalam Kannan[e], Matthew Albertolle[a], Yan Zhou[c,g], Namrata Prasad[a,b,c], Penelope M. Drake[a], Linda C. Giudice[b,c], Steven C. Hall[a,b,c], H. Ewa Witkowska[a,b,c], Germaine M. Buck Louis[d], Susan J. Fisher[a,b,c,f,g,h,i,*]

[a]Sandler-Moore Mass Spectrometry Core Facility, University of California San Francisco, San Francisco, CA, USA
[b]Center for Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA
[c]Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA
[d]Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA
[e]Wadsworth Center, New York State Department of Health, Empire State Plaza, Albany, NY, USA
[f]Division of Maternal Fetal Medicine, University of California San Francisco, San Francisco, CA, USA
[g]The Eli & Edythe Broad Center for Regeneration Medicine and Stem Cell Research, University of California San Francisco, San Francisco, CA, USA
[h]Human Embryonic Stem Cell Program, University of California San Francisco, San Francisco, CA, USA
[i]Department of Anatomy, University of California San Francisco, San Francisco, CA, USA

ARTICLE INFO

Article history:
Received 16 April 2014
Accepted 22 September 2014

Keywords:
Endometriosis
Peritoneal fluid
Urine
Omental fat
Mass spectrometry
Environmental chemicals

ABSTRACT

Endometriosis, ectopic growth of the uterine lining (endometrium), which affects 6–11% of reproductive age women, is associated with pelvic pain and infertility. We investigated the peritoneal fluid (PF), urine and omental fat (OF) proteomes of women with endometriosis vs. individuals with no surgically visualized endometriosis. All participants were enrolled in the NICHD-funded ENDO Study. A two-step proteomic study was performed. The first, a broad survey, employed a semi-quantitative gel LC-mass spectrometry (MS) workflow: SDS PAGE fractionation, trypsin digestion and LC–MS/MS. The results showed sample integrity but failed to detect any differences between women with and without endometriosis. The second step was a quantitative analysis of OF samples. We employed another sample set (n = 30) from women ± disease and isobaric mass-tag (iTRAQ) chemistry to label peptides and 2D LC–MS/MS for protein identification and quantification. Three proteins—matrix metalloproteinase-9, neutrophil elastase, and FAM49B—were significantly lower in abundance in samples from women with endometriosis. Interestingly, neutrophil elastase and FAM49B levels were associated with higher levels of a subset of endocrine disrupting

* *Corresponding author at: Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA.* Tel.: +1 415 476 5297; fax: +1 415 476 5623.
E-mail address: sfisher@cgl.ucsf.edu (S.J. Fisher).

http://dx.doi.org/10.1016/j.jprot.2014.09.015
1874-3919/

chemicals (EDCs) that were previously measured in the same samples. The results of these experiments showed the feasibility of associating endometriosis with changes in the OF protein repertoire and EDC levels.

Biological significance
Endometriosis, pathological growth of the uterine lining, is associated with significant morbidities, including pain and infertility. However, the causes of this common condition are poorly understood. This study determined whether endometriosis was associated with changes in the protein composition of peritoneal fluid, urine and/or omental fat. A protein of unknown function (FAM49B) and two proteinases (metalloproteinase-9, neutrophil elastase) were down regulated in OF samples from women with versus without endometriosis. These findings suggested proteinase imbalances at sites that were distant from the endometriotic lesions. Additionally, FAM49B and neutrophil elastase levels were associated with higher levels of a subset of environmental chemicals that were quantified in the same samples, suggesting other possible associations. Thus, this work generated hypotheses that will be tested in further studies.

1. Introduction

Proteomic signatures lend insights into normal and abnormal processes at cell, tissue and organismal levels. Although genetic and epigenetic profiles yield valuable information, the protein machinery they assemble gives us the best functional insights into the mechanisms underlying biological processes. However, global profiling approaches face the daunting challenge of enormous proteome complexity. To date, mass spectrometry-based techniques have been the primary tools of investigators interrogating the protein repertoire. In this regard, there has been particular interest in the proteomes of human biological fluids as they offer a window into health and disease. Those that are easily obtained—e.g., urine [1], plasma [2] and saliva [3] among others—have been intensely studied as potential sources of biomarkers. In endometriosis research, a rapid, noninvasive technique for diagnosis and follow-up using peripheral biomarkers as surrogates of disease is an active area of investigation (reviewed in [4–6]). For example, urinary enolase I, vitamin D-binding protein, and cytokeratin 19 have been proposed as potential diagnostic biomarkers [7–9], though more work is needed to validate these candidates in larger sample sizes [10]. However, there are several problems associated with proteomic analyses of body fluids, including protein abundances over several orders of magnitude. In addition, the signals of interest are often from a point source that is distal to where the sample is collected, thereby creating the additional problem of dilution [11]. These difficulties have prompted analyses of more proximal samples, such as peritoneal fluid from women with endometriosis [12–14] or the actual cell type of interest such as fat from obese individuals [15].

Endometriosis, which involves endometrial growth outside the uterus, affects 6–11% of all women of reproductive age [16,17]. A primary cause is thought to be transport and subsequent adherence of endometrial cells/tissue to the peritoneal cavity [18]. However, other theories have been proposed, including lymphatic or vascular metastases [19], immunologic deficits resulting in impaired clearance of ectopic endometrium [20] and coelomic metaplasia [21]. Estrogen, which is necessary for the proliferation and survival of endometriotic tissue, is plentiful in the peritoneal cavity with sources including the ovaries, follicular fluid, and the blood [22]. Endometrial implants elicit an inflammatory response, resulting in a host of pathologies including angiogenesis, adhesions, fibrosis, scarring, neuronal infiltration, and anatomical distortion [16,22–24]. As a result, patients with endometriosis may experience mild to severe pelvic pain and/or infertility.

Endocrine-disrupting chemicals (EDCs) are generally defined as substances in the environment, food, and consumer products that interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control of reproduction [25]. Seemingly ubiquitous, endocrine disruptors are present in industrial solvents and lubricants [polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins], pesticides [methoxychlor, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT)], fungicides (vinclozolin), pharmaceutical agents [diethylstilbestrol (DES)] and flame retardants [polybrominated diphenyl ethers (PBDEs)] [25,26]. A wealth of evidence links exposures to EDCs with causation or exacerbation of endometriosis [25,27–33]. For example, pre-natal exposure of mice to bis-phenol A (BPA) results in female offspring with an endometriosis-like condition [30,32]. Investigators have also evaluated phthalate levels in blood plasma collected from women diagnosed with endometriosis vs. age-matched women without disease, finding potential differences [27,29,31,34]. After adjusting for serum lipid levels, gravidity, and tobacco use, four PCB congeners, which are anti-estrogens, imparted a three-fold risk of developing endometriosis in a cohort of 84 women [28]. Furthermore, an evaluation of persistent organic pollutants (POPs) in omental fat (OF) and serum collected from women diagnosed with endometriosis revealed a significant positive association between α-hexachlorocyclohexane levels and endometriosis [33].

In this study, we utilized a two-stage MS-based analysis to investigate the protein repertoire of PF, urine and OF samples that were obtained from women who had been diagnosed with endometriosis and women with no surgically visualized