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Review

Gender proteomics I. Which proteins in non-sexual organs

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ABSTRACT

Differences related to gender have long been neglected but recent investigations show that they are widespread and may be recognized with all types of *omics* approaches, both in tissues and in biological fluids. Our review compiles evidence collected with proteomics techniques in our species, mainly focusing on baseline parameters in non-sexual organs in healthy men and women. Data from human specimens had to be replaced with information from other mammals every time invasive procedures of sample procurement were involved.

Significance: As our knowledge, and the methods to build it, get refined, gender differences need to receive more and more attention, as they influence the outcome of all aspects in lifestyle, including diet, exercise and environmental factors. In turn this background modulates a differential susceptibility to some disease, or a different pathogenetic mechanism, depending on gender, and a different response to pharmacological therapy. Preparing this review we meant to raise awareness about the gender issue. We anticipate that more and more often, in the future, separate evaluations will be carried out on male and female subjects as an alternative – and an upgrade – to the current approach of reference and test groups being 'matched for age and sex'.

1. Introduction

A thorough and systematic assessment of the expression level for the product(s) of each of the protein-coding genes identified in the human genome is being carried out through the construction of a Human Protein Atlas (http://www.proteinatlas.org) as part of the HUPO endeavor (https://www.hupo.org). At the level of organ proteomes, the Atlas database contrasts *testis* and *prostate* to *endometrium*, *ovary* and *placenta*. No data on the contrary are reported about the occurrence of differential regulation of any protein at sites other than gonads and genitals; sex (and age) of tissue donors together with diagnosis are only included in specific histological and Ref-seq data. No sex-specific information is available either from the ProteomicsDB repository, which collects thousands of LC-MS/MS experiments involving human tissues, cell lines and body fluids to provide a draft of the human proteome [1].

Our review aims at filling this gap by compiling evidence on gender-specific differences at the proteomic level throughout organs and tissues. Such differences have been reported for dioichous plants and for many animals in all phyla. We will focus our report on humans and, to a minor extent, on laboratory animals, as their specimens are analyzed under a number of experimental settings as models of physiological and pathological conditions in our species. As we have long ago seen in our investigations [2–4] and as we summarize in Fig. 1, the differences between plasma/serum and urine proteomes between males and females in one of these species, *Rattus norvegicus*, are so obvious that the origin of the specimens can be easily guessed from the spot pattern in a 2-DE slab. Subtler yet significant differences are observed in human body fluids and in some human and animal tissues, as we are going to detail in the following.

Our reference list only contains a little > 100 items. Indeed, on most topics, the reports pointing out gender differences, and proving some features being dependent of sex, amount to a very small fraction of the total of proteomics investigations. In some more cases, mostly dealing with the search for biomarkers of disease, the possible occurrence of gender differences was investigated and eventually ruled out: several features were thus proven independent of sex; to keep our account focused, we do not review these reports but we like to stress that relevance and reliability are equal between evidence in favor or in disfavor of a given conclusion. In most cases, however, the investigated features were made operationally independent of sex through comparison/s between/among sex-matched experimental groups; this was most often the case with difficult to obtain clinical specimens from human patients. This amounts to disregard the influence of sex, if any, and was routine till recently. Indeed, also the endeavor of gender medicine, as aiming at understanding the differences of patho-physiology, clinical signs, prevention and treatment of diseases equally

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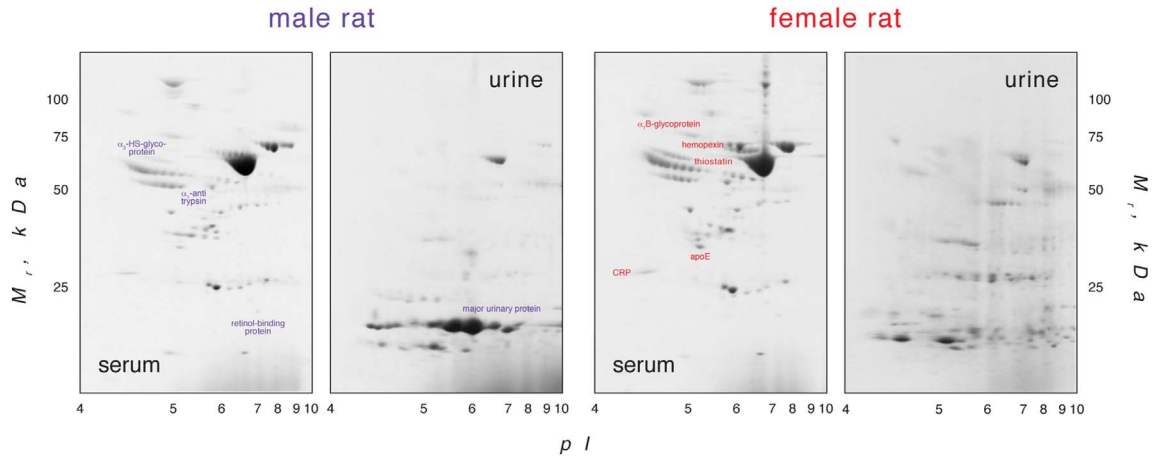

Fig. 1. 2-DE pattern of serum and urine from male (left) and female rats (right) as resolved on a 4–10 NL IPG and on a 7.5–17.5% T PAA gradient (modified from Fig. 3 in [4]). The names of the proteins for which differences are systematically observed between genders are marked; the increase falls below 2-fold in most cases but amounts to orders of magnitude for thiostatin in female serum and for major urinary protein in male urine [2,3].

represented in men and women (while not studying either gender-related diseases or diseases prevalent in one gender) [5] is rather recent and incompletely developed. In the past century, biological and chiefly pharmacological investigation in mammals used to be carried out only in males, as the cyclic changes in females connected with their reproductive function, and the asynchrony of such changes among individuals, was resented as a confounding element. The information collected in this review provides conclusive evidence that such an approach amounts to an unacceptable oversimplification of the biological realm. The limited number of the existing reports, overall and addressing each individual topic; the lack of match for age, genetic background, lifestyle among the reference groups; the extreme variability among the experimental procedures: these are some of the reasons to define the evidence collected so far as a challenge for future endeavor rather than as the end result of past commitment. As we'll see both listing the results in individual areas of investigation and trying to survey the body of present-day knowledge, the state of affairs allows drawing only limited conclusions about recurrent patterns or general trends. This situation urges researchers to go deeper in the assessment of gender proteomics, much beyond the current level of available data, which in many cases are best defined as preliminary.

2. Genes set the scene

2.1. How does it all begin?

In mammals, sex development is a genetically and hormonally controlled process that includes three sequential steps (Jost paradigm [6]). The first such step is the establishment of chromosomal, or genetic, sex (XY or XX) at conception. The second corresponds to gonadal differentiation, towards the formation of either a testis in males or an ovary in females: the former is initiated, at approximately 6 to 7 weeks of human gestation, by the expression of the Y chromosome-linked sex-determining gene called SRY while the latter is the default outcome in the absence of SRY gene products. The third step corresponds to sex differentiation, and involves the development of internal and external reproductive organs and the acquisition of secondary sex characteristics. Male differentiation is controlled by three hormones produced by the testis: Mullerian-inhibiting substance, or anti-Mullerian hormone, testosterone, and insulin-like factor 3; their absence results in female differentiation [7].

Gene SRY encodes a transcription factor that is a member of the high mobility group (HMG)-box family of DNA-binding proteins; because of its function, this protein is named testis-determining factor (TDF). Mutations in the SRY gene give rise to XY females with gonadal dysgenesis (Swyer syndrome); translocation of part of the Y chromosome containing this gene to the X chromosome causes XX male syndrome (http://www.ncbi.nlm.nih.gov/gene/6736). Some hint to the mechanism of action of SRY has been gathered by investigating the effects of its overexpression in two stably transfected lines derived from human testicular embryonic cell carcinoma NT2/D1 cells [8]. Comparing protein amounts by 2-DE demonstrated down-regulation of many chaperone proteins together with up-regulation of laminin, which is important for Sertoli cell differentiation, tubular formation and testis development. Transcriptomic analysis through microarray technology detected higher levels of mRNAs coding for many zinc finger proteins but lower levels for cellular growth factors. Cell growth analysis found inhibition of S or G_2/M transit with arrest of the cell cycle and inhibition of cellular proliferation. In a different perspective, the first stages of gonadal differentiation in mouse embryos, at the ages of 11.5 and 12.0 days *postcoitum*, were monitored by 2-DE [9]. This extensive survey, carried out by 2D LC-MS/MS, led to the recognition of a few proteins (7 over a total of 1000) as specific to testis, both adult and embryonic, *vs* other organs – these proteins are likely to have testis-specific roles throughout the life of the organism – and of a larger group (81 for testis and 171 for ovary) as specific to embryonic *vs* adult gonads – these proteins, also expressed in adult organs other than gonads, are likely to have a specific function during organogenesis. In line with the relative quiescence of the ovarian development pathway compared with the morphologically active testis pathway, male samples contained all of the identified proteins whereas female samples contained only a fraction (60%) of them; proteins common to both sexes are likely to have a generic cellular or developmental function within the gonads, whereas proteins uniquely identified in the testis (the remaining 40%) could be involved in regulating embryonic testis differentiation and development. No protein was uniquely identified in the ovary.

2.2. Genetic equity through dosage compensation?

While in the most ancient organisms sex is determined by environmental or social variables, chromosome-based mechanisms are at work in less ancient species, including ours. Through evolution, differentiation of dimorphic sex chromosomes from a pair of autosomes occurred in a number of independent instances, resulting in XY (or X0) systems, with heterogametic males, and in ZW (or Z0) systems, with heterogametic females.

The first event in Y chromosome differentiation in our lineage [10] was a mutation in the SRY-box 3, or SOX3, gene that - not < 180 million years ago - shifted its function from the regulation of embryonic development to a critical determinant of maleness in the form of the