

Cellular/molecular features and sex differences

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Men and women, despite being subject to the same pathologies, present significant differences regarding: onset, incidence, progression, response to treatments and prognosis. To be noted, the state of health or illness can be influenced not only by health aspects related to sex but also by socio-economic and cultural factors. However, the sum of these determinants leads to impressive differences that have still to be clarified in terms of features and mechanisms. In fact, some years ago, the American National Institute of Health Recommendations for Research clearly suggested the need to monitor the differences and similarities for all human diseases affecting both sexes; to develop studies dealing with gender differences starting at the cradle and to promote sex and gender research at the cellular level, i.e. aimed at investigating the mechanisms underlying the disparities. On these bases and following the specific requests of many granting agencies asking for the inclusion of sex differences in both preclinical and clinical studies, several research groups have started to investigate either the biological or the socio-cultural features of many human diseases and the molecular mechanisms, if any, underlying the sex/gender differences mentioned above.

On these bases the Italian National Institute of Health (ISS) recently created a Reference Center, i.e. a Department, with the specific aim to help the NHS in the development of this field both in terms of the research on gender/sex specific determinants and the implications for health policy. In particular, the mission of this structure, composed of about 60 people with different skills, will be involved in the following activities: Promotion of education and training activities; Correct evaluation of prevention-related approaches in both sexes (i.e. life styles, nutrition, risk assessment); Identification of representative sex-specific biomarkers, useful for diagnosis, prognosis and predictive evaluations; Effects of sex/gender-specific disparities evaluated as responses to therapies; Analyses of pathogenetic mechanisms underlying sex/gender differences associated with diseases; Participation in defining new guidelines and recommendations taking into account gender-specificities; Role of sex/gender differences in infective disease susceptibility and response to vaccinations;

Data collection and epidemiological analysis on disease evolution, morbidity and mortality taking into account sex and gender differences; Gender-oriented clinical and therapeutic paths; Compliance with the recent Italian law dealing with the introduction of gender medicine in clinical practice.

As concerns research activities carried out at the ISS, recent data can be summarized as specified below.

Cellular mechanisms

It has been shown that XX and XY cells of different histotypes (e.g. endothelial, epithelial, fibroblasts, lymphocytes) clearly respond to an exogenous stress, e.g. an inflammatory stress, in different ways. In particular: i) the basal redox state (XX cells show a higher antioxidant power); ii) susceptibility to oxidative stress, which can result in alterations of intracellular key molecules such as cytoskeleton; iii) under the same stress conditions (drugs, prooxidants, etc.) XY cells more easily undergo apoptosis, whereas XX cells undergo autophagic cytoprotection or senescence; iv) different responses to drugs, e.g. chemotherapy or antiviral drugs; v) similar data are now emerging for non-nucleated cells (e.g. platelets); vi) either genetic (XY, XX chromosomes) or epigenetic (microRNA) mechanisms can be involved; vii) Response to steroid hormones could be involved.

Cells and estrogens

Estrogen receptors (ERs) are expressed by XX and XY cells without any difference. Both receptors are being studied: ER-alpha and ER-beta. Localization of ERs can be nuclear, mitochondrial, and at the cell surface. Ligand of ER-alpha is considered to be anti-apoptotic. Ligand of ER-beta is also pro-apoptotic. The ER-alpha/ER-beta ratio is critical. Some cells, e.g. lymphocytes, constitutively express ERs at the cell surface, other cells, e.g. cardiomyocytes, express these receptors under stress only. The receptors are able to trigger an intracellular pathway, i.e. they are functional receptors. We hypothesize that, despite nuclear receptors, the membrane re-

ceptors can provide the cells with a prompt response, within minutes allowing cell modifications, survival or death depending on their role in the organism. It means that membrane ER-alpha activation could trigger lymphocytes survival and movement machinery whereas in cardiomyocytes could ignite cell survival.

Sex chromosomes

The transcription of genes present in both *X chromosomes* would lead to a dangerous overexpression of their products, which is avoided by the inactivation of one of the two. In almost all mammals, the chromosome to be deactivated is randomly selected from the two available. Different cells of the same organism can have a different X active (and, consequently, the expression of different alleles for genes present in heterozygosis on the two chromosomes). The Y chromosome contains very few genes (about 200, of which 72 code for proteins). As proposed by Art Arnold, beyond its roles in testis determination and spermatogenesis, the Y chromosome could be essential for male viability, and in the phenotypic differences between the sexes, both in health and disease (Art Arnold).

Epigenetics

Epigenetics refers to the changes that influence the phenotype without altering the genotype. An epigenetic signal is an inheritable change that does not alter the nucleotide sequence of a gene but its activity. MicroRNAs (miRNAs) are small endogenous molecules of single-stranded non-coding RNA. These are polymers encoded by nuclear eukaryotic DNA of about 20-22

nucleotides and mainly active in the regulation of gene expression at the transcriptional and post-transcriptional levels. The X chromosome contains 10% of all the miRNAs present in the genome (more than 100) whereas only 2 miRNAs, to date, have been identified on the Y chromosome. The impact of this scenario in the XX and XY cell homeostasis has recently been investigated (Carè et al., 2018).

Mitochondria

Mitochondrial dynamics is impaired in senescent cells and a large number of aging associated diseases. This impairment involves excessive mitochondrial fission, resulting in mitochondrial structural changes and dysfunction, and cell damage. Inhibitors of mitochondrial fission have been proposed as a therapeutic strategy for diseases with oxidative stress and mitochondrial dysfunction. Since a significant difference in XX and XY cells senescence and vulnerability mechanisms has been demonstrated, a series of analyses has been performed aimed at identifying if mitochondria could play a role. We found that mitochondrial remodelling in cells from males could improve the resistance of these cells to exogenous stressors. In other words, the inhibition of mitochondrial fission in cells from males leads to an improvement of the mitochondrial network leading to a sort of "feminization" of the cells with a consequent increased resistance to injury and death.

All in all, these data demonstrate the need to improve our knowledge on the sex disparity at the cellular level in order to improve our understanding of the sex-dependent pathogenetic mechanisms and the consequent development of a gender-pharmacology and, more in general, an appropriate gender/sex-specific medicine.

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