

## Male and female cells: same stress, different response

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The unknown causes of the well-established differences between males and females, with regard to the incidence and response to treatment in several different conditions, is the new challenge in biomedical research. In fact, despite the evident gender- and sex-related differences confirmed by the growing literature data, there are still relatively few studies conducted at the cell and molecular levels in order to reveal the mechanisms responsible for sexual dimorphism.

“Gender medicine”, that studies the influence of biological (defined by sex) and socio-economic and cultural (defined by gender) differences on each person’s health and state of illness, precisely emphasizes how the two sexes are equal in terms of right to health, but not equivalent in terms of disease manifestations.

Many international studies, both observational and meta-analyses, “photograph” gender disparity in health and in illness. Autoimmune diseases are a representative example of conditions that show a dramatic gender difference in terms of incidence, since they predominantly affect the female sex. In addition, very recent studies clearly show that also cardiovascular diseases should be considered in their gender specificity.<sup>1,2</sup> Similarly, in the oncology field, significant data are emerging on gender differences in the incidence and response to treatments, with particular reference to the innovative cancer immunotherapies.<sup>3-5</sup> In fact, precision medicine, at least in this field, is far ahead. Nevertheless, the studies analyzing the mechanisms underlying these differences are still inadequate, and in Europe and in US many researchers are developing and validating appropriate experimental *in vitro* and *in vivo* models to study the baseline mechanisms of the reported sex and gender differences, particularly those related to pathogenetic mechanisms and pharmacology.

Here, we refer to a research that goes in this direction, trying – that is – to identify the molecular mechanisms underlying the physiological sex differences highlighted in literature. In fact, the comprehension of those mechanisms could bring enormous advantages, both in the identification of possible gender-related risk factors and in the development of gender-specific therapies.

A group of researchers from the ISS’s Gender Reference Center, in collaboration with researchers from the Uni-

versity of Bologna and the CNR of Rome, recently published in the international journal *Cell Death and Disease* the results of a study showing differences in behavior between female and male cells isolated from the context and cultured *in vitro*.<sup>6</sup> In particular, the researchers were able to identify some molecular components directly involved in the different response of masculine (XY) and feminine (XX) cells to mitochondria-mediated stress.

In general, male (XY) cells respond to stressors (oxygen radicals, pro-inflammatory cytokines, drugs) by undergoing a programmed death (apoptosis), while female cells (XX), in response to the same stress, activate survival mechanisms (autophagy) and become resistant to apoptotic cell death.<sup>7</sup>

In addition to the different genetic structure of the cells (XX vs XY), the sexual dimorphism in the cell response to external stimuli could be correlated also to the different expression of epigenetic regulators – including microRNAs (miRs) – between the two sexes.

MicroRNAs are short sequences of endogenous and non-coding RNA that, by regulating the expression of genes, are able to influence different cell processes, such as differentiation, proliferation, metabolism and cell death. In many metabolic, degenerative and neoplastic diseases, alterations of the expression levels of specific microRNAs have been observed. Although a causal role of these changes in the pathogenesis is not always evident, microRNAs expression changes may play an important role as pathological biomarkers.

MicroRNAs are encoded also on sex chromosomes. In particular, the X chromosome contains 10% of all the microRNAs present in the genome, while the Y chromosome contains only 4.

Female cells have two X chromosomes, while male cells have one X chromosome, and one Y chromosome. To maintain a balance in the number of genes and proteins, one of the two X chromosomes is inactivated in female cells. However, some portions of the X chromosome, corresponding to approximately 10%, escape the inactivation. In particular, it is estimated that approximately 10% of the X chromosome content escapes inactivation.

In light of these premises, starting from a bioinformatic analysis, some microRNAs located in those regions

of the X chromosome that escape inactivation were selected. Some of these have been assumed to play a role in the epigenetic regulation and de-regulation of XX and XY cellular homeostasis. In particular, the level of miR548am-5p was experimentally quantitated in human female cells (skin fibroblasts), where its expression was found to be five times higher than in male cells of the same origin.<sup>7</sup> MiR548am-5p targets several genes involved in mitochondria-mediated death processes, including Bax and Bcl2, two key proteins impelling the cell towards survival or death. The working hypothesis that the high level of miR548am-5p in female cells could be responsible for the greater resistance to mitochondrial stress was further verified experimentally. Researchers induced an overexpression of miR548am-5p in XY fibroblasts, thereby observing a significant reduction in the apoptosis following treatment with a mitochondria-mediated death inducer. On the other hand, the down-regulation of miR548am-5p in XX cells by transfection with the miR antagonist (anti-miR548am-5p) increased the susceptibility of female cells to apoptosis. These experiments indicated a direct role of this microRNA in the resistance to the mitochondria-mediated cell death observed in “female cells”.

The above reported data do not absolutely show a greater resistance of the XX cells compared to the XY cells in response to any type of stress, but they do show a greater resistance of the female cells to death induced by the activation of the mitochondrial pathway.

The differences identified between male and female subjects at cell level could play an important role in many of the diseases that show significant differences between men and women in terms of incidence, diagnosis, prognosis and/or response to therapy.

Many questions still remain open. For example: could what was observed by researchers in skin fibroblasts have a general value? That is, can it also apply to other cell types with different functions, such as lymphocytes, cardiomyocytes or hepatocytes? To answer this and other questions it will be mandatory to extend the study to other cytotypes, while also investigating in detail the interaction between miR548am-5p and its target mRNA and genes.

However, these data, although not conclusive, could pave the way for the use of microRNAs as biomarkers for those diseases that present a different incidence or a different clinical outcome in the two sexes, as well as innovative sex-specific therapeutic targets, in view of a desirable development of increasingly more specific and personalized therapies, in a context of precision medicine.

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