OPEN

Citation: Cell Death and Disease (2014) 5, e1121; doi:10.1038/cddis.2014.73

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News and Commentary

# Taking into account the gender issue in cell death studies

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*Cell Death and Disease* (2014) **5,** e1121; doi:10.1038/cddis.2014.73; published online 13 March 2014

The work by Jog and Caricchio,[1] recently published in *Cell Death and Disease*, hypothesized an inherent difference in the cell death program between the sexes. In particular, they demonstrate that bone marrow-derived macrophages from male and female mice are prone to different types of cell death. Necrosis in cells from males is PARP-1 dependent, and inhibition of PARP-1 triggers the apoptotic cell death program. By contrast, in cells from females, cell death is PARP-1 independent. In addition, estrogens have a survival activity in female cells only, independent of estrogen receptor (ER) expression. Thus, estrogens and PARP-1 seem to represent two important determinants of the sex bias in cell death. Therefore, these authors propose that targeting cell death on the basis of sex will lead to tailored and better treatments for each gender. This is in line with a great challenge of the recently developed field of investigation dealing with the biology of sex differences and, more in general, with the so-called gender medicine.[2] The main scope of this research field is aimed at the development of a made-to-measure pharmacologic approach that, taking into account gender disparity, could provide new and successful therapeutic strategies.[3,4] We perfectly agree with these assumptions. The basis of this theory stems, in fact, on some recently published papers dealing with the role of 'cell sex' (i.e., freshly isolated cells with XX or XY chromosomes) in the susceptibility to cell death induction. In these studies, it was underscored that several key differences exist between XX and XY cells in this regard. Main features that have been suggested to display a gender disparity at cellular level are the following: (i) the basal redox state[5]; (ii) the response to oxidative imbalance[6,7]; (iii) the susceptibility to undergo apoptosis and anoikis[8,9]; (iv) the susceptibility to undergo autophagy.[10] These insights derived from several *in vitro* studies carried out in human and mouse cells of different histotypes including, for example, human endothelial cells and mouse neurons.[11,12] A key factor in this scenario is represented by the ER signaling pathway. ERs are very important regulators of a plethora of cellular events, including apoptosis and autophagy, and can be expressed by both XX and XY cells at similar levels. Briefly, at least two types of ERs have been described ($\alpha$ and $\beta$), and the preponderance of one of these ERs over the other might change the impact of estrogen activity.[10,13,14] As mentioned above, Jog and Caricchio[1] demonstrate both *in vitro*, in bone marrow-derived macrophages, and *in vivo*, in renal cells, that, under stress conditions, estrogen rescues female cells from death, whereas it shifts cell death to apoptosis in male cells. Hence, the response of males and females to estrogen appeared different. We have to add further insights in this scenario, thanks to a previously hypothesized activity of estrogen in determining the cell fate of freshly isolated human endothelial and smooth muscle cells from males and females. Our findings were referred to as the cell-sex-associated implication of RLIP76 in the maintenance of cell homeostasis.[11,15] This is a Ral effector GTPase-activating protein that catalyzes ATP-dependent transport and extrusion from the cell of anionic conjugates, including reduced glutathione (GSH) conjugates, such as GSH-4-hydroxy-t-2,3-nonenal (GS-HNE), leukotrienes, and weakly cationic compounds. In particular, the 4-HNE is of great relevance in this issue. It is an end product of lipid peroxidation that induces oxidative stress, causes apoptosis, activates several signaling pathways, and is conjugated with GSH.[16] By lowering GS-HNE, RLIP76 contributes to the maintenance of cell homeostasis. By exogenously inducing a mild oxidative stress, that is, mimicking an inflammatory state, the extruding activity of this pump resulted in enhancement. However, a gender disparity was detectable: the pump appeared to have a key role in the control of redox homeostasis mainly in female cells, whereas its role in male cells appeared as negligible.[11] In fact, we found that female cells expressed constitutively higher levels of cell surface RLIP76 and estrogen supply can further increase the expression levels of RLIP76 in female cells rescuing them from death, whereas in cells from males this effect of estrogen was lacking.[11]

Hence, the literature data from our group and from other authors are partially in accord with the work by Jog and Caricchio, even if different cell histotypes have been investigated.[1,11,12,17] In particular, evidence has been reported that: (i) neurons from males, under starvation conditions, more readily undergo cell death, whereas neurons from females mobilize fatty acids, accumulate triglycerides, form lipid droplets, and survive longer[12]; (ii) vascular cells from females are more resistant to $H_2O_2$-induced apoptosis than those from males, and this could be partially explained by RLIP76 expression and function[11]; (iii) estrogens amplify this

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difference, increasing RLIP76 expression only in female cells[11]; and (iv) finally, at variance, macrophages from male and female mice do not differ in their susceptibility to $H_2O_2$-induced apoptosis or necrosis but estrogens rescue female cells from death, whereas they shift cell death program to apoptosis in male cells.[1] Hence, even these cells, that *per se* produce reactive oxygen compounds for their professional killing activity, seem to only partially differ from the other cell types. This complex scenario is schematically reported in Figure 1.

In conclusion, from a general point of view, literature agrees with the idea that cells from females are more resistant to stressors, including cell injury and death insults, than cells from males, surviving better to the microenvironmental stress. Estrogen appears as a relevant actor in this scenario representing a key survival signal for XX cells. Hence, the research priority in the field of gender medicine should be to understand how XX and XY cells could respond to ER signaling. In fact, it now appears clear that male and female cells present a different ability to modulate the activity or expression rate of different molecular targets in response to estrogens (e.g., PARP-1, ER, RLIP76) and this can result in a gender disparity of the adaptive behavior of cells from males and females. However, it is well known that even in the pediatric age, where the impact of hormones is very low, a gender disparity in terms of pathogenetic mechanism and progression of metabolic, inflammatory, (cardio)vascular, neoplastic or neurodegenerative diseases can be observed.[18] This clearly suggests that other important genetic, epigenetic or environmental factors have to be considered.[19,20]

To note, all the studies reported above deal with freshly isolated cells: cell sex 'memory' can, in fact, be lost after few *in vitro* passages so that all cells look alike and gender disparity get vanished. Thus, the analysis of cell fate in a gender perspective, although mandatory, is a matter of availability of robust experimental models. In our opinion and in line with Jog and Caricchio, we think that the

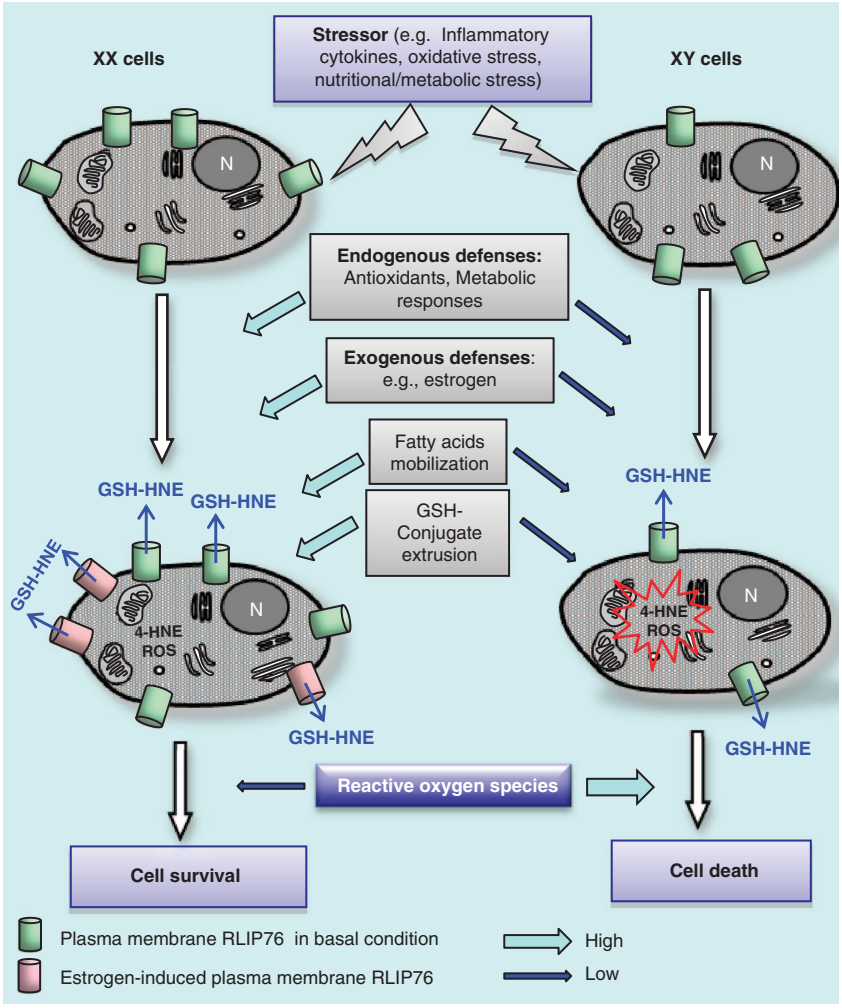


**Figure 1** Different response of XX and XY cells to different stressors as emerging from experimental data obtained from human and murine cells of different histotype. RLIP76, a cell surface protein that in vascular cells catalyzes the extrusion from the cell of reduced glutathione conjugates, has an important role in detoxification of intracellular 4-hydroxynonenal (4-HNE)-catalyzing ATP-dependent transport of GS-HNE. At basal level, XX cells show more efficient endogenous defenses, higher surface expression of RLIP76 and a greater metabolic plasticity than XY cells. After stress, XX cells display a more efficacious mobilization of fatty acids and GSH-conjugate extrusion that determine a significantly minor accumulation of reactive oxygen species. In addition, estrogens represent a trophic factor that can induce an increased surface expression of RLIP76 in XX cells but not in XY cells. Thus, XY cells are more susceptible to stress-induced death than XX cells

development of this field, that is, the biology of sex differences, could provide, in the long run, a gender-biased pharmacological approach and novel therapeutic strategies that could improve the appropriateness of therapy.

## Conflict of Interest

The authors declare no conflict of interest.

**Acknowledgements**. This study was partially supported by AIRC (9998, 11505); Arcobaleno Onlus; Peretti Foundation.

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