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Our knowledge on the pathogenetic mechanisms could lead to:

- A gender-specific prevention
- A gender-specific diagnosis
- A gender-specific therapy
Pathogenetic Mechanisms and gender

- Several gender differences can be explained considering the activity of hormones

HOWEVER:

- Studies with animals (es. four core genotypes*) demonstrated that not all gender disparities are due to hormones
- Studies on genetic diseases (e.g. studies on mitochondrial diseases) have demonstrated gender differences
- Several diseases (both communicable and non-communicable) show gender differences in pediatric diseases.
- Differences at cell level (XX and XY cells)


(*) mice in which sex chromosome complement (XX vs. XY) is unrelated to the animal's gonadal sex. The four genotypes are XX gonadal males or females, and XY gonadal males or females.
Autophagy and senescence

Cell programs

- Proliferation
- Cell death (e.g. by apoptosis)
- Autophagy and senescence
Cell programs:

- apoptosis
- autophagy
Apoptosis in human pathology

Cancer
- Colorectal
- Glioma
- Hepatic
- Neuroblastoma
- Leukaemia and lymphoma
- Prostate

Autoimmune diseases
- Systemic Lupus Erythematousus
- Autoimmune Lymphoproliferative Syndrome
- Thyroid diseases

Inflammatory diseases
- Bronchial Asthma
- Inflammatory intestinal disease
- Pulmonary inflammation

Viral Infections
- Adenovirus
- Baculovirus

Neurodegenerative diseases
- Alzheimer’s disease
- Amyotrophic Lateral Sclerosis
- Parkinson’s disease
- Huntington’s disease
- Epilepsy

Haematologic diseases
- Aplastic anaemia
- Myelodysplastic Syndrome
- T CD4⁺ lymphocytopenia
- G6PD deficiency

Cardiovascular diseases
- Myocardial infarction
- Cerebrovascular accident
- Atherosclerosis
- Vasculitis
- Diabetes

Viral Infections
- AIDS
AUTOPHAGY

Metabolic dysfunction

Elimination of Aberrant Structures

Type II Programmed Cell Death

Aging

Defense Against Pathogens

Cancer cell survival

Development and Cell Differentiation

MHCII presentation of cytosolic Ag
Autophagy in human pathology

- Cancer
- Autoimmune diseases
- Viral infections
- Bacterial infections
- Neurodegenerative diseases
- Cardiovascular diseases
Gender cytopathology (peripheral blood)

Cytopathological alterations of RBC, or of other peripheral blood cells, can show a gender disparity.

This gender disparity can provide useful diagnostic or prognostic tools in certain human diseases.

This gender disparity can contribute to the pathogenesis or the progression of human diseases.
Diametro di un capillare 5µm
Diametro di un eritrocita 7µm
100 billions RBC/day

RBC e fibrina
The microenvironment can shift erythrocytes from a friendly to a harmful behavior: they can exert a pathogenetic role in vascular diseases

- Erythrocytes are a potential component in atheromatous lesions and thrombus formation.
- Sex hormones may influence the fatty acid composition of human erythrocyte membrane.
- Erythrocytes undergoing apoptosis (eryptosis) exposing PS at their surface tend to aggregate. This can lead to changes of rheological properties of RBC per se and to a pro-oxidant activity of erythrocytes towards other cells, including RBC-RBC RBC-platelets and RBC-endothelial cells.

Yunoki et al. 2012; Lhoner et al. 2013, Malorni and Minetti 2008
Erythrocytes show a peculiar form of apoptosis

Lucantoni et al. Antiox Redox Sign 2006
The microenvironment can shift erythrocytes from a friendly to a harmful behavior

(Minetti & Malorni 2007; Minetti et al. 2008)
**Table 1.** Patients’ characteristics. Significant differences are in bold. Data are the mean ± S.D. of 56 MetS patients (31 male and 25 female) and 40 HD (22 male and 18 female).

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>56 MetS (M=31 F=25)</th>
<th>40 HD (M=22 F=18)</th>
<th>P-VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Mass Index (Kg/m²)</td>
<td>31.98±4.84</td>
<td>21.05±2.01</td>
<td>0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>112.7±14.98</td>
<td>71.79±6.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134.57±17.7</td>
<td>120.63±8</td>
<td>0.03</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>84.13±9.37</td>
<td>74.95±5.68</td>
<td>0.001</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td><strong>122.71±34.87</strong></td>
<td>63.84±9.38</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>197.62±38.83</td>
<td>178.39±20.23</td>
<td>0.15</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>125.4±36.08</td>
<td>114.27±28.51</td>
<td>0.31</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>44.41±8.47</td>
<td>47.09±8.8</td>
<td>0.78</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>144.8±72.28</td>
<td>118.94±48.96</td>
<td>0.26</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>12 (50%)</td>
<td>4 (21%)</td>
<td>0.60</td>
</tr>
<tr>
<td>Family history of Diabetes</td>
<td>13 (54%)</td>
<td>5 (26%)</td>
<td>0.80</td>
</tr>
<tr>
<td>Currently smokers</td>
<td>5 (21%)</td>
<td>12 (63%)</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Echocardiography parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>52.46±6.16</td>
<td>59.68±2.82</td>
<td>0.001</td>
</tr>
<tr>
<td>SIV (mm)</td>
<td>11.58±0.92</td>
<td>8.74±1.28</td>
<td>0.0001</td>
</tr>
<tr>
<td>PP (mm)</td>
<td>11.13±0.90</td>
<td>9.21±1.22</td>
<td>0.001</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>126.78±31.19</td>
<td>115.00±18.93</td>
<td>0.05</td>
</tr>
<tr>
<td>LVESV (ml)</td>
<td>58.57±17.20</td>
<td>40.05±6.32</td>
<td>0.002</td>
</tr>
<tr>
<td>LVM-I (g)</td>
<td>114.25±16.84</td>
<td>77.36±31.24</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Carotid echo-color-Doppler parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCA Sx (mm)</td>
<td>1.25±0.36</td>
<td>0.73±0.22</td>
<td>0.0001</td>
</tr>
<tr>
<td>ICA Sx (mm)</td>
<td>1.68±0.73</td>
<td>0.67±0.26</td>
<td>0.001</td>
</tr>
<tr>
<td>CCA Dx (mm)</td>
<td>1.30±0.39</td>
<td>0.56±0.28</td>
<td>0.001</td>
</tr>
<tr>
<td>ICA Dx (mm)</td>
<td>1.96±0.79</td>
<td>0.66±0.27</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Senescent RBCs (Glic A+)

Apoptotic RBCs (AV+)

Median values

<table>
<thead>
<tr>
<th></th>
<th>CTR</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Senescence and cryptosis modify RBC rheology (Straface et al. 2010)

Modulations in autophagy have been associated with diseases of the heart, including cardiomyopathies, cardiac hypertrophy, ischemic heart disease, heart failure, and ischemia–reperfusion injury


Upregulated autophagy protects cardiomyocytes from oxidative stress-induced toxicity. Autophagy. 2013 Mar 1;9(3):328-44.

Matarrese et al. Induction of autophagy protects cardiomyocytes from stress-induced arrhythmia upregulating membrane estrogen receptors alpha, submitted
17-β-estradiol

**Pro-apoptotic activity**
High concentrations (over 10nM)
- Lymphocytes
- Leukemic cells
- Monocytes
- Thymocytes
- Endothelial cells from coronary aorta
- VSMC from thoracic aorta

**Anti-apoptotic activity**
Low concentrations (below 10nM)
- T and B cells
- Endothelial cells
- VSMC
- Cardiomyocytes
- Neurons
- Platelets
Estrogen receptors at the cell surface (mER)

Under oxidative imbalance (e.g. mimicking an inflammatory state) cell responses seem to be:

i) the up-regulation of mERα, but not mERβ, at the cell surface associated with

ii) rapid functional signals (ERK phosphorylation and p38 dephosphorylation) leading to

iii) apoptosis hindering and autophagic cytoprotection, i.e. to cell survival.
What are the mechanisms underlying gender disparity
## Cultured cells in preclinical studies

<table>
<thead>
<tr>
<th>Cell “name”</th>
<th>Cell type</th>
<th>Sex</th>
<th>Isolation date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jurkat</td>
<td>Lymphoid cells</td>
<td>male</td>
<td>1970</td>
</tr>
<tr>
<td>CEM</td>
<td>Lymphoid cells</td>
<td>female</td>
<td>1964</td>
</tr>
<tr>
<td>Hep-2</td>
<td>epidermoid carcinoma</td>
<td>male</td>
<td>1952</td>
</tr>
<tr>
<td>Hela</td>
<td>epidermoid carcinoma</td>
<td>female</td>
<td>1951</td>
</tr>
<tr>
<td>U937</td>
<td>Lymphoid cells</td>
<td>male</td>
<td>1974</td>
</tr>
<tr>
<td>NCI-H292</td>
<td>mucoepidermoid carcinoma</td>
<td>female</td>
<td>1985</td>
</tr>
<tr>
<td>Vero</td>
<td>Kidney (monkey)</td>
<td>unknown</td>
<td>1962</td>
</tr>
<tr>
<td>SH-SY5Y</td>
<td>neuroblastoma</td>
<td>female</td>
<td>1970</td>
</tr>
<tr>
<td>PC12</td>
<td>pheochromocytoma (rat)</td>
<td>male</td>
<td>1976</td>
</tr>
</tbody>
</table>
# The memory of the cells: the birth of “cell sex”

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Species</th>
<th>N. of passages (with “memory” of their sex)</th>
<th>Investigation tools mainly in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblasts</td>
<td>Human, rat, mouse</td>
<td>About 10</td>
<td>Cardiovascular, autoimmune</td>
</tr>
<tr>
<td>Vascular Smooth Muscle Cells (VSMC)</td>
<td>Human, rat, mouse</td>
<td>About 10</td>
<td>Cardiovascular, Gastroenterology</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>Mainly murine</td>
<td>About 5</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>Resting Lymphocytes</td>
<td>human</td>
<td>-</td>
<td>Immune system and inflammatory diseases</td>
</tr>
<tr>
<td>Platelets</td>
<td>human</td>
<td>-</td>
<td>Hematological, Neurodegenerative diseases</td>
</tr>
<tr>
<td>Red Blood cells</td>
<td>human</td>
<td>-</td>
<td>Hematological</td>
</tr>
<tr>
<td>Freshly isolated cancer cells</td>
<td>human, mouse</td>
<td>10-15</td>
<td>Experimental chemotherapy</td>
</tr>
<tr>
<td>Mouse Embrio Fibroblasts (MEFs)</td>
<td>mouse</td>
<td>10-20</td>
<td>Mechanisms of drug toxicity</td>
</tr>
<tr>
<td>Keratinocytes</td>
<td>human</td>
<td>10-12</td>
<td>Dermatology</td>
</tr>
<tr>
<td>Neuronal cells</td>
<td>mouse, rat</td>
<td>-</td>
<td>Neurodegenerative</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>human</td>
<td>About 10</td>
<td>vascular</td>
</tr>
</tbody>
</table>
Reactive Oxygen Species are capable of activating a series of cell functions, e.g. transcription factors.

But, also, free radicals, mainly ROS, are known to induce a number of intracellular lethal and sublethal alterations including:

- DNA damage
- Oxidative alterations of proteins
- Oxidative alterations of (membrane) phospholipids
- Apoptosis as well as autophagy
Cytoskeleton
Female Male

Control

Under stress

Actin filaments

Cells with "cross-links" of actin filaments (%)

Basal UVB

Male Female
“male cells” are more susceptible to death than “female cells”...

![Graph showing cell death percentages for male and female cells with different treatments.](chart)

- Control
- UVB 24h
- UVB 48h
- UVB 72h

*Female vs. Male*

![Immunostaining images showing cell morphology.](images)
...whereas female cells easier undergo senescence.
Effects of oxidative stress on VSMCs: “male cells” are more susceptible

“female cells” have a reduced ROS production and/or “more” antioxidant defenses than “male cells”

Note different “basal” levels

Straface et al. 2010
Male cells typically exhibit a higher rate of autophagy induction compared to female cells. Under stress, female cells develop a more pronounced autophagic (protective) response.
Conclusions

• Freshly isolated vessel cells, e.g. smooth muscle and endothelial cells (3-8th passage), maintain a sort of “memory” of their sexual origin. In particular:

  • i) a different “basal” redox state.

  • ii) a different susceptibility to stress.

  • iii) this susceptibility appears associated with a different fate: “male cells” appear more “apoptotic-prone” whereas “female cells” survive better and appear more “autophagy-prone” cells

A higher ability of “female cells” to adapt to environmental changes (behavioral plasticity)?
Some recent reviews published by our group