On the role of autophagy in human diseases: a gender perspective

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Abstract

Cytopathological features of cells from males and females, *i.e.* XX and XY isolated cells, have been demonstrated to represent a key variable in the mechanism underlying gender disparity in human diseases. Major insights came from the studies of gender differences in cell fate, *e.g.* in apoptotic susceptibility. We report here some novel insights recently emerged from literature that are referred as to a cytoprotection mechanism by which cells recycle cytoplasm and dispose of excess or defective organelles, *i.e.* autophagy. Autophagy and related genes have first been identified in yeast. Orthologue genes have subsequently been found in other organisms, including human beings. This stimulated the research in the field and, thanks to the use of molecular genetics and cell biology in different model systems, autophagy gained the attention of several research groups operating to analyse the pathogenetic mechanisms of human diseases. It remains unclear, however, whether autophagy can exert a protective effect or instead contribute to the pathogenesis of important human diseases. On the basis of the growing importance of sex/gender as key determinant of human pathology and of the known differences between males and females in the onset, progression, drug susceptibility and outcome of a plethora of diseases, the idea that autophagy could represent key and critical factor should be taken into account. In the review, we summarize our current knowledge about the role of autophagy in some paradigmatic human diseases (cancer, neurodegenerative, autoimmune, cardiovascular) and the role of 'cell sex' differences in this context.

Keywords: cell ● autophagy ● gender ● pathogenesis ● hormones

Introduction

In the cell biology, the term autophagy defines the catabolic process regulating the degradation of a cell's own components through the lysosomal machinery [1, 2]. Autophagy is a genetically regulated process that plays an important homeostatic role in

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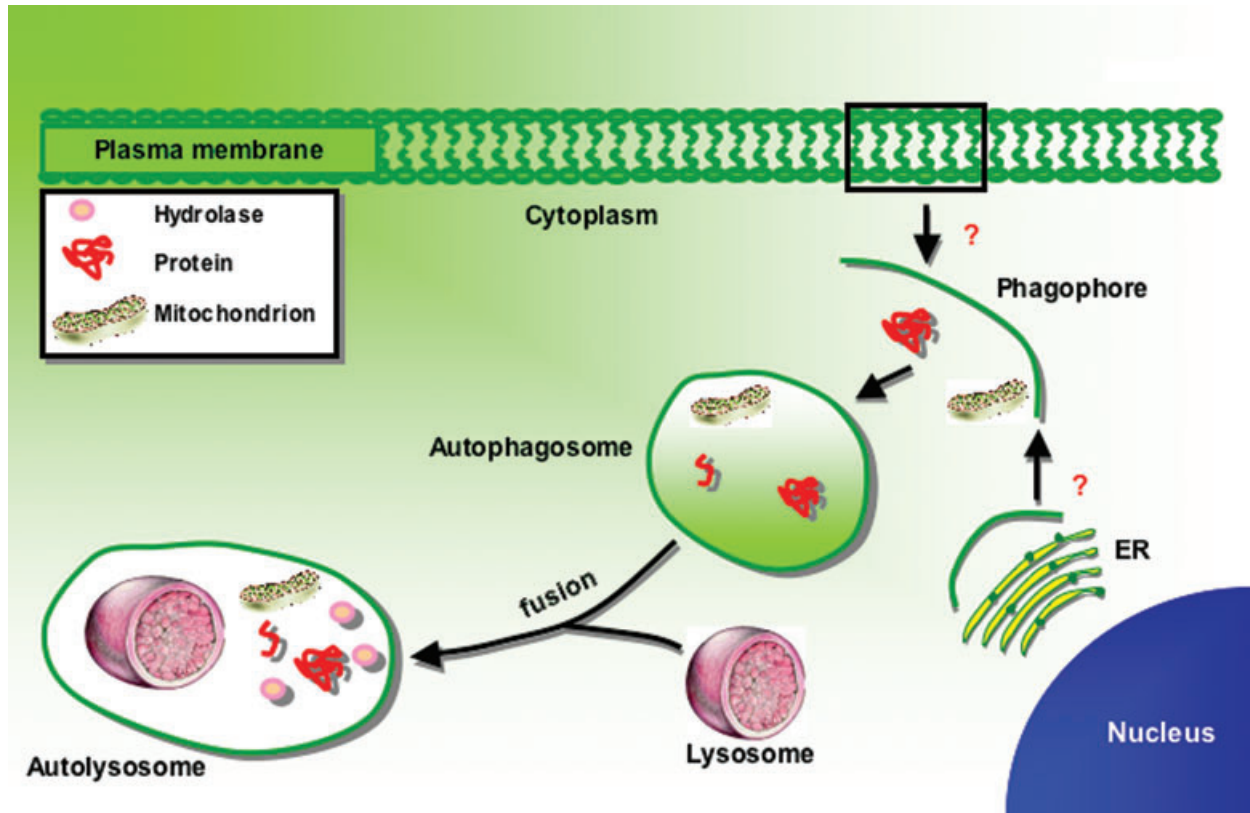Fax: +3906 49903691
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Fig. 1 The autophagic pathway. In macroautophagy, the cargoes are sequestered within a unique double-membrane cytosolic vesicle, an autophagosome. Sequestration can be either non-specific, involving the engulfment of bulk cytoplasm, or selective, targeting specific cargoes such as organelles or proteins. The autophagosome is formed by expansion of the phagophore, but the origin of the membrane is unknown; probably, it may origin from plasma membrane or ER. Fusion of the autophagosome with a lysosome provides proteases, *e.g.* hydrolases, to the formed organelle. Lysis of the autophagosome inner membrane and breakdown of the contents occurs in the autolysosome and the resulting macromolecules are released back into the cytosol through membrane permeases.

cells, preserving the balance between the synthesis, degradation and subsequent recycling of cellular components [3]. The term 'autophagy', derived from the Greek and meaning 'eating of self', was first coined by Christian de Duve over 40 years ago, and was largely based on the observed degradation of mitochondria and other intracellular structures within lysosomes [4]. In recent years the scientific world has 'rediscovered' autophagy, and major contributions to our molecular understanding of the physiological significance of this process came from a number of laboratories [5–7].

Autophagy is an evolutionarily conserved and strictly regulated lysosomal pathway that degrades cytoplasmic material and organelles. It is activated during stress conditions such as metabolic stress, amino acid starvation, unfolded protein response or viral infection [8, 9]. It has been suggested that autophagy, limiting necrosis and inflammation, inducing cell cycle arrest and preventing genome instability, could prevent tumorigenesis [10]. Autophagy has also recently been shown to be required for key aspects of the senescent cell phenotype [11], which is known to

be anti-tumorigenic. However, as a cell survival mechanism, other authors have argued that autophagy may promote intrinsic drug resistance and tumour cell adaptation to dysmetabolic stress [12].

Although the importance of autophagy is well recognized in mammalian systems, many of the mechanistic breakthroughs in delineating how autophagy is regulated and executed at the molecular level have been made in yeast (*Saccharomyces cerevisiae*) [13]. Currently, 32 different autophagy-related genes (Atg) have been identified by genetic screening in yeast and, significantly, many of these genes are conserved in slime mould, plants, worms, flies and mammals, emphasizing the importance of the autophagic process in responses to starvation across phylogeny [5]. There are three defined types of autophagy: macro-autophagy, micro-autophagy and chaperone-mediated autophagy, all of which promote proteolytic degradation of cytosolic components at the lysosome (Fig. 1).

Macro-autophagy delivers cytoplasmic cargos to the lysosome through the intermediary of a double membrane bound vesicle,

Table 1 Some relevant human diseases displaying gender differences (in terms of incidence, symptoms and/or prognosis) are reported. The role of autophagy is also outlined

Disease	Gender differences	Role of autophagy	References
Cancer	In CRC, NSCLC, HCC, melanoma and chronic lymphocytic leukaemia.	Prevent tumorigenesis but an increased autophagy may be associated with chemioresistance more than with cell death.	[1], [2], [6], [10–11], [28], [39], [41], [138–140]
Neurodegenerative and psychiatric diseases	In Parkinson disease, Huntington disease, autism, schizophrenia, Alzheimer disease and Niemann–Pick syndrome.	A decreased or defective autophagy is pathogenetic.	[6], [50–52], [55], [59], [85]
AID	In systemic lupus erythematosus, scleroderma, rheumatoid arthritis, primary biliary cirrhosis, autoimmune thyroid disease, Sjogren's syndrome and multiple sclerosis.	Involved in the promotion of MHC class II presentation and breakdown of tolerance.	[73], [75–76], [81]
Cardiovascular diseases	In several vascular and heart diseases, including diabetes, thrombosis, hypertension, coronary heart disease, metabolic syndrome, cardiac fibrosis.	Autophagy confers cytoprotection under metabolic and ischemic stress.	[85–87], [89], [91], [95–96], [98], [102], [135]

referred as to an autophagosome, which fuses with the lysosome to form an autolysosome. In micro-autophagy, by contrast, cytosolic components are directly taken up by the lysosome itself through invagination of the lysosomal membrane. Both macro- and micro-autophagy are able to engulf large structures through both selective and non-selective mechanisms. In chaperone-mediated autophagy, targeted proteins are translocated across the lysosomal membrane in a complex with chaperone proteins (such as heat shock chaperone-70) that are recognized by the lysosomal membrane receptor lysosomal-associated membrane protein 2A, resulting in their unfolding and degradation [14]. After induction by a metabolic stress signal, such as nutrient deprivation, the first step in macro-autophagy is the formation of an autophagosome. A flat membrane cistern elongates and wraps itself around a portion of cytoplasm or a specific cargo, forming a double membrane bound autophagosome. This membrane cistern is called phagophore, or isolation membrane. The origin of this membrane cistern is still under debate. Probably, it may origin from plasma membrane or endoplasmic reticulum (ER). Autophagosomes next receive lysosomal constituents, such as lysosomal membrane proteins and proton pumps, by fusion with late endosomes or multivesicular bodies. Finally, autophagosomes fuse with lysosomes (Fig. 1) [13]. The general features and the methodological approaches to analyse autophagy have recently reviewed by Mizushima *et al.* [15].

Very important is the role that autophagy pathway plays in some diseases. In this regard we will report here some of the most paradigmatic human diseases where autophagy, or disturbances of autophagy, have been suggested to play a role. Moreover, when available, information as concerns the gender/sex issue has also been considered. The development of gender medicine, *i.e.* of a medicine taking into account gender/sex differences in diagnosis and therapy of human diseases, has in fact been encouraged and promoted by the World Health Organization and by several scientific institutional and governmental agencies. Several human diseases clearly display gender disparities in terms of pathogenetic mechanisms, age of onset, progression and cure appropriateness. Hence, investigations taking into account the gender issue appear as a key and novel research field. An important point in this regard concerns terminology. As a general rule, the term 'sex' is used to distinguish male or female patients according to the reproductive organs and functions that derive from the chromosomal complement. This is distinct from the term 'gender', used to refer to human being's self-presentation as males or females considered from a 'social' point of view [16]. In fact, at cellular level, the terms sex or gender appear quite inappropriate. In our opinion we can use 'cell sex' (referred as to XX and XY cells) to indicate cells obtained from male or female animals or human beings. However, the term gender could paradoxically be used also in the case of cells and their pathology, often due to changes in their microenvironment or in cell-cell interactions, *i.e.* from a social point of view. In this review, we briefly analyse the implication of this rediscovered pathological feature of the cell, autophagy, in a gender perspective.

Autophagy and disease

Males and females show significant differences in the prevalence of many major diseases that have important inflammatory components to their aetiology. They include autoimmune diseases (AID), some cancers, metabolic and vascular diseases and are largely considered to reflect the actions of sex hormones on the susceptibility to inflammatory stimuli [17]. Some examples of these diseases will briefly be discussed in the light of the recent implication of autophagy as pathogenetic determinant or therapeutic target and taking into account hormone effects (Table 1).
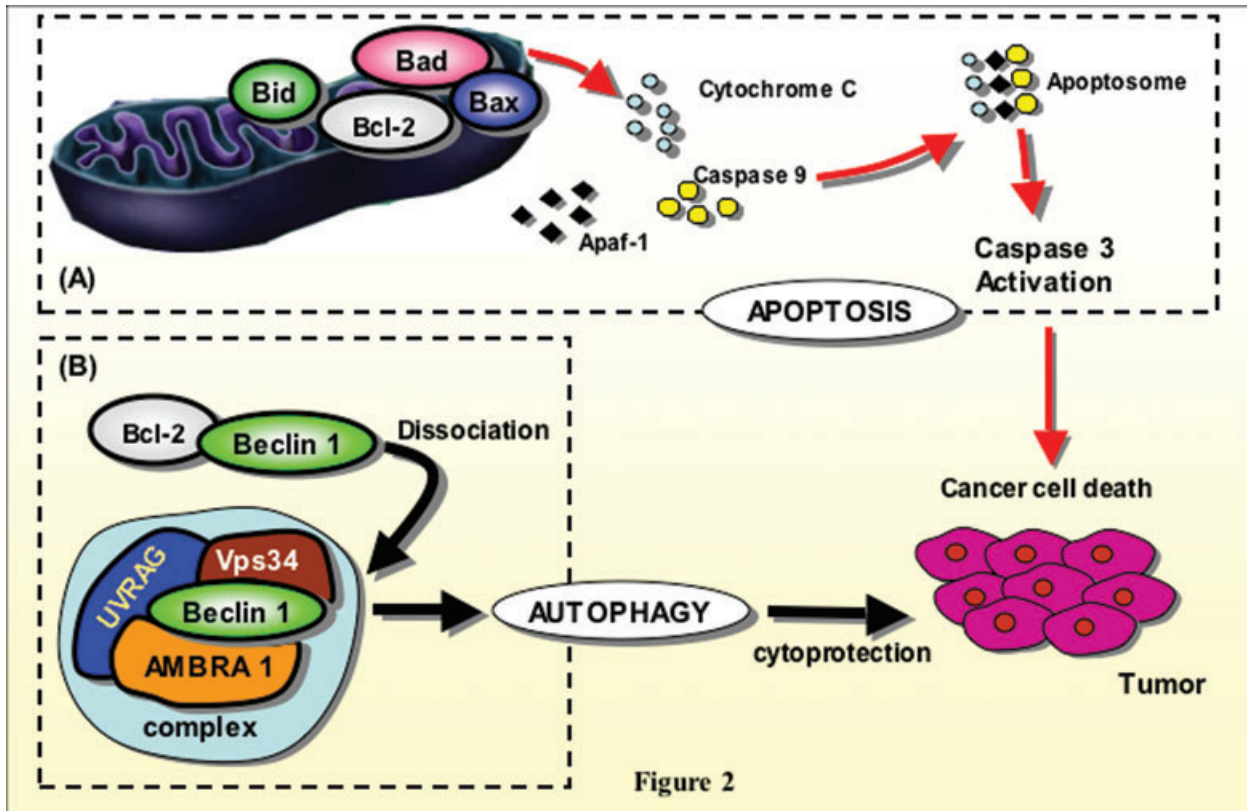
Fig. 2 Autophagy, apoptosis and Bcl-2 in cancer. Schematic representation of the role of Bcl-2 during apoptosis (**A**) and autophagy (**B**) in tumour cells. (**A**) The pro-apoptotic Bcl-2 proteins Bad, Bid and Bax may reside in the cytosol and translocate to mitochondria following death signalling, where they promote the release of cytochrome c inducing apoptosis. (**B**) Bcl-2 may dissociate from Beclin-1 promoting autophagy. Both apoptosis and autophagy may play a role in cancer.

Cancer

Autophagy and cancer

It has been suggested that autophagy could prevent tumorigenesis [10] but, also, that impaired autophagy could contribute to cancer development [6, 18]. This could occur *via* a de-regulation of cell growth and/or *via* a decreased cell death. In fact, autophagy can benefit the progression of the tumour because it can provide nutrients during starvation [6, 18], can sustain cell metabolism and can limit chromosomal instability [19]. These findings suggest that autophagy inhibition, rather than stimulation, might be beneficial in treatment of advanced cancer.

Several studies refer to the role of autophagy in gender-specific cancer cell models, *e.g.* in female cancer. For example, Beclin-1/Atg6, is monoallelically deleted in a large proportion of human breast and ovarian cancers: mice with heterozygous deletion of Beclin-1 have less autophagy and more tumours than control mice [20, 21]. Conversely, overexpression of Beclin-1 in a breast cancer cell line increases autophagy and decreases the growth and tumorigenicity of these cells [22]. Beclin 1-VPS34 is a complex

that plays a crucial role in the induction of the autophagic process by generating PtdIns(3)P-rich membranes. Several cofactors, such as Ambra1, ATG14 and UVRAG (UV radiation resistance-associated gene protein), are necessary for Beclin 1 complex activity. Some of these autophagy-promoting cofactors such as UVRAG and Ambra 1 have also been suggested to act as tumour suppressors (Fig. 2) [23, 24]. On the other hand, binding of the proto-oncogenic proteins B-cell lymphoma 2 (Bcl-2) or Bcl-XL to Beclin-1 inhibits autophagy [25]. Accordingly, knockout of Bif-1, also part of the Beclin-1 complex, significantly enhances the development of spontaneous tumours in mice [26]. In addition to the Beclin-1 complex, other tumour suppressors also enhance autophagy: phosphatase and tensin homologue protein, a tumour suppressor, is a phosphatase that decreases the concentration of class I PI3K product and enhances autophagy [27].

Gender differences in cancer

As concerns gender, few data are available so far. Examples of cancer with documented gender disparity can be seen in colorectal cancer (CRC), in hepatocarcinoma (HCC) and in non-small cell

lung cancer (NSCLC) and melanoma (Table 1). In fact, recent population studies highlight the importance of gender differences in CRC [28]. *In vitro* studies have demonstrated both growth promotional and inhibitory effects of oestrogen on oestrogen receptors in normal colonic mucosa and CRC cells [29, 30]. Progressive hypermethylation of the Cytosine-phosphate-Guanine islands (regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide) of the promoter region of the oestrogen receptor gene occurred in normal colonic mucosa with age, resulting in reduced receptor expression [31]. These studies suggested that oestrogen receptors have tumour suppressor ability and inhibition of their transcription, affected by methylation, deregulated colonic growth. Furthermore, addition of oestrogen suppressed colonic cell growth. From this, it is possible to suggest that hypermethylation may be associated with declining oestrogen and aging, although the exact interactions are unknown. Molecular studies have demonstrated the selective protection of oestrogen in CRC through interaction with oestrogen receptor β (ERβ), microsatellite status and Cytosine-phosphate-Guanine methylation. Oestrogen levels affect ERβ expression, which is associated with the development of microsatellite-instability high cancers [31]. Pathways involving hypermethylation and mismatch repair genes have been proposed [32, 33]. However, the exact molecular mechanisms remain elusive. Ongoing research in this area is imperative. Given these known associations of oestrogen with CRC, the potential clinical implications associated with the decrease in hormone replacement therapy following the Women's Health Initiative Study may result in changing epidemiology of CRC in women, and close observations in this evolving area are crucial [34, 35].

Autophagy, gender disparity and cancer

As concerns autophagy in this context, it has been demonstrated that 2-methoxyestradiol (2-ME) is able to induce apoptosis as well as autophagy. This evidence pointed out that 2-ME could be considered as a promising tool against colon carcinoma [36]. Recently, sulforaphane, a kind of isothiocyanate, has been demonstrated to induce autophagy in colon cancer cells as a protective mechanism [37]. These authors thus claim for the inhibition of autophagy to potentiate anticancer therapy. Accordingly, the combination therapy with chloroquine, used as autophagy inhibitor, has been suggested to represent a novel therapeutic modality to improve the efficacy of 5-fluorouracil-based chemotherapy, possibly inhibiting autophagy-dependent resistance to chemotherapy [38]. It has been suggested that gender could in fact represent a key challenge in future studies on the management of CRC [39].

Two further examples of gender-associated cancer of interest in the present context could be NSCLC and HCC [40, 41]. As concerns the first, a gender disparity has been described and such disparity is apparently due to a variety of mechanisms, ranging from genetic and epigenetic differences, to gender-specific lifestyle as well as to behavioural causes and, clearly, to sex hormones activity. It has been found that certain anticancer agents, *e.g.* therapeutic antibody that blocks epidermal growth factor receptor function, can act by promoting an association between

beclin 1 and hVps34, which was inhibited by overexpression of Bcl-2, suggesting that autophagy could protect cancer cells from the pro-apoptotic effects of antibodies [42]. In some instance, improved survival was associated with female gender, which appears as more sensitive to these therapeutic strategies [43]. Regarding HCC, showing a male/female ratio averaging between 2:1 and 4:1, the role of autophagy has been assessed and therapeutic strategies aimed at increasing autophagy, *i.e.* using rapamycin and derivatives, have been suggested to exert beneficial effects in patients [44–46]. Moreover, the levels of autophagy protein Beclin-1/Atg6 have been suggested to have a prognostic significance [47]. As concerns the possible implication of hormones in this scenario a recent work suggest that sexually dimorphic actions of glucocorticoids could play a role [17]. Moreover, some further insight also derived from experimental studies. For instance, the implication of the oestrogen-derived anti-angiogenic compound 2-ME, as pro-autophagic agent has been suggested [48] but the possible implication of metabolic hormones in autophagy induction was described since many years (reviewed in Klionsky, 2007 [13]). Finally, pro-survival signalling mediated by the androgen receptor (AR) has been suggested to contribute to prostate carcinogenesis: maintained AR signalling promotes temporary adaptation to cellular stress and may contribute to hindering prostate tumour cell death [49].

Neurodegenerative diseases

Gender differences in neurodegenerative diseases

In the brain, from a physiological point of view, biological sex differences are of great importance. To maintain homeostasis, if the neurological system is challenged by external factors, such as stress and disease, different organizations in circuitries in male and female brains will respond. Conditions that differ markedly in their prevalence, progression and/or severity between the sexes include Parkinson's disease, attention deficit/hyperactivity disorder, autism and schizophrenia, all of which show a greater prevalence in men and involve the midbrain dopoaminergic systems. In contrast, in Alzheimer's disease, involving cognitive brain regions such as the hippocampus, postmenopausal women fare worse than men [50]. This implies fundamental differences between men and women in the underlying pathophysiology, which in turn has implications for responsiveness to treatments [51, 52]. Furthermore, many age-related neurodegenerative diseases are characterized by the accumulation of ubiquitin-positive protein aggregates in affected brain regions. These misfolded, aberrant proteins can disrupt neuronal function and cause neurodegeneration. A better understanding of the neurobiological basis for sex differences in brain disorders is, therefore, a key goal for improving therapies for conditions for which current treatments have limited success.

Role of autophagy in neurodegenerative diseases

Autophagy is necessary for the clearance of aggregate-prone proteins that are toxic especially for post-mitotic cells like neurons

[6] (Table 1). A good example is represented by clearance of aggresome in some neurodegenerative diseases such as Huntington disease and familial Parkinson's disease. Autophagy was shown to enhance the clearance of misfolded or aberrant proteins such as huntingtin, mutant τ, synphilin 1 and α-synuclein, but not aminoacyl-transfer ribonucleic acid synthetases-interacting multifunctional protein and mutant desmin [53]. Enhanced autophagy in animal models of these diseases improves clearance of the aggregated proteins and reduces the symptoms of neurodegeneration [54]. In the same vein, Alzheimer's disease is characterized by the accumulation of extracellular amyloid plaques in the brain. These plaques consist of aggregated β-amyloid (Aβ) peptide. Autophagic compartments containing both amyloid precursor protein and Aβ accumulate in dystrophic neurons in Alzheimer brain [55, 56] and autophagic compartments were identified as a major reservoir of intracellular Aβ in the brain of Alzheimer patients and mouse models [57, 58]. A recent study challenges the idea that autophagy contributes to the pathogenesis of Alzheimer's disease *via* changes in Beclin-1 expression [58]. A further example of the involvement of autophagy in neurodegeneration could be considered the Niemann–Pick neurodegenerative disease. This is lipid storage disorder characterized by a disruption of sphingolipid and cholesterol trafficking caused by mutations in either of two genes, npc1 and npc2 (Niemann–Pick C genes). The disease produces cognitive impairment, ataxia and death, often in childhood. Cells deficient in npc genes show increased expression of Beclin-1 and LC3-II (light chain-3 protein), the autophagosome-specific form of LC3, suggesting that autophagy is induced [59]. Further works also claim that increased autophagy may be harmful for neurons in patients with Niemann–Pick disease [60, 61].

Gender differences and hormones

Without doubt, oestrogen has been pinpointed as a critical protective factor in females that gives them the advantage in diseases prevalent in men, whereas its rapid decline after menopause may forfeit this advantage. In theory, oestrogen holds great clinical potential for central nervous system disorders because of its proven neuroprotective and neuroactivating properties [62–64]. Therefore, it is important to highlight that the sex differences in brain organization is critical to develop optimal therapies for the common disorders that differentially affect men and women.

Because autophagy and its disturbances are well-documented pathogenetic determinants of several neurodegenerative diseases and on the basis of the well-known differences in the epidemiological data collected worldwide on these diseases, the need for further gender-biased analyses on this matter appears as mandatory. In fact, at the best of our knowledge specific gender- biased studies are still lacking, a part from a very interesting study carried out in mice on endothelin-1/vascular endothelial growth factor signal peptide-activated receptor (DEspR) that plays a role in neuroepithelium and neural tube differentiation. The authors found in male mice only that DEspR haploinsufficiency impaired hippocampus-dependent visuospatial and associative learning, non-inflammatory spongiform changes and neuronal vacuolation consistent with autophagic cell death. Conversely, DEspR$^{\pm}$ females exhibited better cognitive performance than wild-type females and showed absence of neuropathological changes. This elegant study highlights gender-associated cerebral neuronal vulnerability to autophagic dysregulation [65]. A further example could be that of hippocampal neural stem cells derived from the adult rat brain that following insulin withdrawal displayed features of autophagy, including increased expression of Beclin-1 and the type II form of LC3 [66].

Autoimmune and inflammatory diseases

Gender differences in autoimmune diseases

The human immune system manifests some degree of sexual dimorphism with basic immune responses differing between females and males. In general terms, women have an enhanced antibody production and increased cell-mediated responses following immunization whereas men produce a more intense inflammatory response to infectious organisms [67, 68]. Furthermore, women have higher CD4$^+$ T-cell counts than men which contributes to an increased CD4/CD8 ratio, higher levels of plasma immunoglobulin M (IgM), and greater Th1 cytokine production [69, 70]. Some recent findings demonstrating the presence of non-nuclear oestrogen receptors at the cell surface of various lymphocyte subsets clearly disclose a new scenario in the gender-associated modulation of immune system [71]. In fact, these receptors appear as functional and capable of activating cell function.

Autophagy and autoimmunity

Lysosomal degradation products are displayed by major histocompatibility class II molecules to CD4$^+$ T cells to mount adaptive immune responses. It has recently been shown that autophagy substrates can also give rise to major histocompatibility complex (MHC) class II ligands. The regulation of autophagy may be beneficial in various disease settings in order to enhance adaptive immune responses or to reduce autoimmunity [72]. However, more in general, as stated by Deretich [73], autophagy plays many parts (Table 1): (*i*) in its most primeval manifestation, it captures and digests intracellular microbes, (*ii*) it is an antimicrobial output of Toll-like receptor (TLR) response to pathogen-associated molecular patterns and (*iii*) it is an effector of Th1-Th2 polarization in resistance or susceptibility to intracellular pathogens. As a regulator of immunity, autophagy plays a multitude of functions: (*i*) it acts as a topological inversion device servicing both innate and adaptive immunity by delivering cytosolic antigens to the lumen of MHC II compartments and cytosolic pathogen associated molecular patterns to endosomal TLRs, (*ii*) it is crucial in T-cell repertoire selection in the thymus and control of central tolerance, (*iii*) it plays a role in T and B cell homeostasis and (*iv*) it is of significance for inflammatory pathology.

Implication of autophagy in AID: paradigmatic examples

AID include more than 70 different disorders. These disorders manifest a wide variability in terms of targeted tissues, age of onset and response to immunosuppressive treatments. The one feature that is shared by the majority of these conditions, however, is the predominance in the female sex, with over 80% of patients with AID being women [74, 75]. The most striking sex differences in AID are observed in Sjogren's syndrome, systemic lupus erythematosus, primary biliary cirrhosis, autoimmune thyroid disease and scleroderma in which 80% of the patients are women. On the other hand, rheumatoid arthritis, multiple sclerosis and myasthenia gravis have a lower female prevalence but still 60–75% of the patients are women. A third group, which includes inflammatory bowel diseases and immune-mediated (type 1) diabetes, is characterized by a female: male ratio that is approaching 1:1 with a slight predominance of the male sex [76]. Finally, very few autoimmune disorders, such as primary sclerosing cholangitis, are characterized by male predominance [74].

As concerns autophagy and its possible implication in AID, several points recently emerged from literature. For instance, recent advances in the study of Atg molecules provide strong evidence that Atgs influence both the host defence response and the regulation of inflammation. Consistently, it has been proposed that the compromised function of autophagy regulators results in the development of immune-related disease, for example the Crohn's disease [77, 78]. Therefore, it would be of interest to assess the therapeutic effects of strategies to increase autophagic function, such as a hypocaloric diet and autophagy inducing drugs, in the treatment of these diseases. Paradigmatic in this issue is the work of Perl and coworkers [79]. These authors found that rapamycin, an autophagy inducer, can be an effective treatment in both murine lupus models and human systemic lupus erythematosus. This is based on the increased knowledge of the role of mammalian target of rapamycin (mTOR) signalling in autophagy throughout the immune system. Finally, autoimmune disorders, cell injury (apoptosis/autophagy), redox balance and gender differences appear as closely connected [80]. In fact, activation, proliferation and death of cells of different histotype, including blood and vascular cells, are under control of oxidative balance and are key players in AID pathogenesis and progression. However, cells from male and female appear characterized by a huge series of differences in terms of reactive oxygen species (ROS) production and oxidative stress susceptibility. Because oxidative imbalance has been associated with autophagy regulation and progression [81], the search in this field will probably provide innovative information in a next future.

The involvement of hormones

AID are considered to reflect the actions of sex hormones on the susceptibility to inflammatory stimuli. However, inflammation reflects a balance between pro- and anti-inflammatory signals, and investigation of gender-specific responses to the latter has been neglected. Glucocorticoids are the primary physiological anti-inflammatory hormones in mammals, and synthetic derivatives of these hormones are prescribed as anti-inflammatory agents, irrespective of patient gender. In fact, Duma *et al.* [18] showed the possibility that sexually dimorphic actions of glucocorticoid regulation of gene expression may contribute to the dimorphic basis of inflammatory disease by evaluating the rat liver, a classic glucocorticoid-responsive organ. In this study, a comparison has been performed among the number of genes involved in inflammatory disorders between sexes revealing 84 additional glucocorticoid-responsive genes in the male. This finding suggested that the anti-inflammatory actions of glucocorticoids are more effective in males [18]. These gender-specific actions of glucocorticoids in liver were substantiated *in vivo* with a sepsis model of systemic inflammation [82].

Cardiovascular diseases

Gender disparity in cardiovascular diseases

Gender in heart and vascular diseases has widely been investigated since many years [83, 84] (Table 1). In particular, the Framingham study investigated into the evolution of cardiovascular disease suggesting the presence of a gender difference in the pathogenetic and progression determinants detectable in men and women. For instance, women were found to outlive men and to experience fewer atherosclerotic cardiovascular events, with an incidence lagging behind that in men by 10 to 20 years [84]. Briefly, it was recognized that women typically develop heart disease later than men and with a better prognosis [85]. From animal studies we learned that there are clear differences in the degree and/or the type of pathological hypertrophy in response to cardiac insults between males and females [86]. Male spontaneously hypertensive rats developed more cardiac hypertrophy and left ventricular dysfunction than females. Subsequent heart failure also occurred earlier in males than females [87]. Similar findings were reported in response to pressure overload (aortic-banding) [88]. Comparable gender differences have also been reported in human beings, though the data are generally less conclusive owing to confounding factors (*e.g.* treatments, lifestyle), the relatively small sample sizes and the limited number of studies in patients with pure pressure overload (*i.e.* in the absence of coronary heart disease) [84, 89].

The molecular mechanisms underlying these gender dimorphisms are complex and are still not well understood [90, 91]. In general, pre-menopausal women tend to be protected against cardiovascular disease compared with age-matched men, but this protection is abolished following menopause. Thus, it has been suggested that oestrogen has protective properties and activation of signalling cascades downstream of oestrogen may explain gender-related differences in the heart [92, 93]. The sex steroid hormones (oestrogen, progesterone and testosterone) and their respective receptors are thus thought to mediate, at least in part, gender differences in the heart [85, 91].

Autophagy and heart diseases

Although altered autophagy has been observed in various heart diseases, including cardiac hypertrophy and heart failure, it

remains unclear whether autophagy plays a beneficial or detrimental role in these diseases [94, 95]. In the heart, autophagy is important for the turnover of organelles at low basal levels under normal conditions and it is up-regulated in response to stresses such as ischemia/reperfusion and in cardiovascular diseases such as heart failure (Table 1). As mentioned earlier, tissue-specific deletion of *ATG5* in the heart causes cardiac hypertrophy and contractile dysfunction [96]. In addition, increased levels of ubiquitinated proteins and abnormal mitochondria are found, especially after treatment with pressure overload or β-adrenergic stress. This suggests that autophagy is needed in the heart to ensure the availability of sufficient energy substrates and to control cardiomyocyte size and global cardiac structure and function. In fact, autophagy appears to play a protective role in cardiomyocytes: enhancing autophagy by Beclin-1 overexpression reduces Bax activation and protects against ischemia/reperfusion injury in cardiac HL-1 cells [97]. A decrease in autophagy at the hypertrophied state facilitates cardiac hypertrophic response. However, cardiac hypertrophic response is similar between cardiac-specific *ATG*5-deficient mice and the control mice after thoracic transverse aortic constriction [98, 99], suggesting that autophagy does not play a role in regulating the cardiomyocyte hypertrophy induced by pressure overload or that its function in the hypertrophic process is compensated by the action of other hypertrophic signalling mechanisms [100]. Thus, the role of autophagy in cardiac hypertrophy remains to be fully elucidated.

A further point concerns cardiomyopathy. In patients with terminal heart failure, secondary to ischemic cardiomyopathy or dilated cardiomyopathy, cellular degeneration with granular cytoplasmic ubiquitin inclusion was detected [101]. In human failing hearts with idiopathic dilated cardiomyopathy, the prevalence of autophagic, apoptotic and necrotic cells have also been observed [102]. Coming back to the animal models, dead and dying cardiomyocytes showing characteristics of autophagy have been observed in murine models [103]. Cardiomyocytes obtained from a genetic model of cardiomyopathic hamsters contain typical autophagic vacuoles, including degraded mitochondria, glycogen granules and myelin-like figures [104]. However, the question remains as to whether autophagy is a sign of failed cardiomyocyte repair or is a suicide pathway for the failing cardiomyocytes.

Several studies have also been conducted in experimental models, either *in vivo* or *in vitro*, in order to characterize the mechanisms underlying autophagy in the heart. For example, mice deficient for a member of a family of membrane glycoproteins involved in the protection, maintenance and adhesion of the lysosome, the lysosomal-associated membrane protein 2A, show excessive accumulation of autophagic vacuoles and impaired autophagic degradation of long-lived proteins, resulting in cardiomyopathy [105, 106]. However, several further actors seem to play a role in autophagy pathway in heart disease. For instance, it has been shown that oxidative stress, ER stress and changes in the ubiquitin–proteasomal system are intimately involved in the regulation of autophagy in the heart, removing damaged organelles, such as mitochondria, and maintaining ER homeostasis [107, 108]. Conversely,

pro-apoptotic factors can be released from the damaged mitochondria, leading to apoptotic cell death [109]. In particular, in the absence of autophagy, the accumulation of polyubiquitinated proteins may be responsible for increased ER stress, and determine cell death by apoptosis [96]. Hence, the balance between autophagy and apoptosis appears as a critical point. In fact, many of the autophagosomes can contain mitochondria in the adult rat myocardium under severe stress such as ischemia/reperfusion suggesting that excessive autophagic activity induced by severe stimuli can destroy a large fraction of the cytosol and organelles and release apoptosis-related factors, leading to cell death [96]. Indeed, characteristics of autophagy, apoptosis and necrosis can also be simultaneously observed in failing hearts [102].

Several signalling pathways that are induced by common cellular stressors regulate both autophagy and apoptosis. ROS not only trigger apoptosis but are also essential for autophagy and specifically regulate ATG4 activity [110, 111]. ROS produces damaged proteins and organelles and lipid peroxidation in mitochondria, thereby promoting autophagy [112, 113]. However, ROS may also stimulate autophagosome formation through direct oxidative modifications of the autophagic machinery [113]. Members of the beclin1 and Bcl-2 family could serve as a point of cross-talk between the autophagic and apoptotic pathways. Beclin-1, primarily localized at ER [114], was originally identified as a Bcl-2-interacting protein [115]. Bcl-2 inhibits Beclin-1/dependent autophagy in yeast and mammalian cells and cardiac Bcl-2 transgenic expression also inhibits autophagy in murine heart cells [116]. During cardiac reperfusion phase there is an increased expression of Beclin-1/ATG6 in the heart [117]. Recently, inhibition of mTOR by Everolimus has been demonstrated to represent a potential therapeutic strategy to limit infarct size and to attenuate adverse left ventricular remodelling after myocardial infarction [118]. Altogether these studies indicate that constitutive cardiomyocyte autophagy is required for protein quality control and normal cellular structure and function under the basal state. Accumulation of abnormal proteins and organelles, especially mitochondria, may directly cause apoptosis and cardiac dysfunction. Autophagy and apoptosis (and their cross-talk) appear thus as two key pathways in the definition of cardiomyocyte survival or death, in turn instructing cardiac integrity and function. However, because autophagic triggering is normally due to metabolic stress and/or nutrient deprivation it is conceivable that ischemic damage could represent a paradigmatic example *in vivo* of what has been observed *in vitro* in isolated cells.

Hormones, gender and cardiovascular diseases

Whether autophagy could play a role in gender differences detected in heart disease is still largely unknown. However, several studies evaluated the possible implication of hormones in the modulation of autophagy and some experimental studies at cellular level suggest that further gender-biased specific analyses appear as mandatory (see below). However, it is well known that sex hormones affect body fat distribution and also impact on cardiovascular system. Because obesity represents a high-risk factor
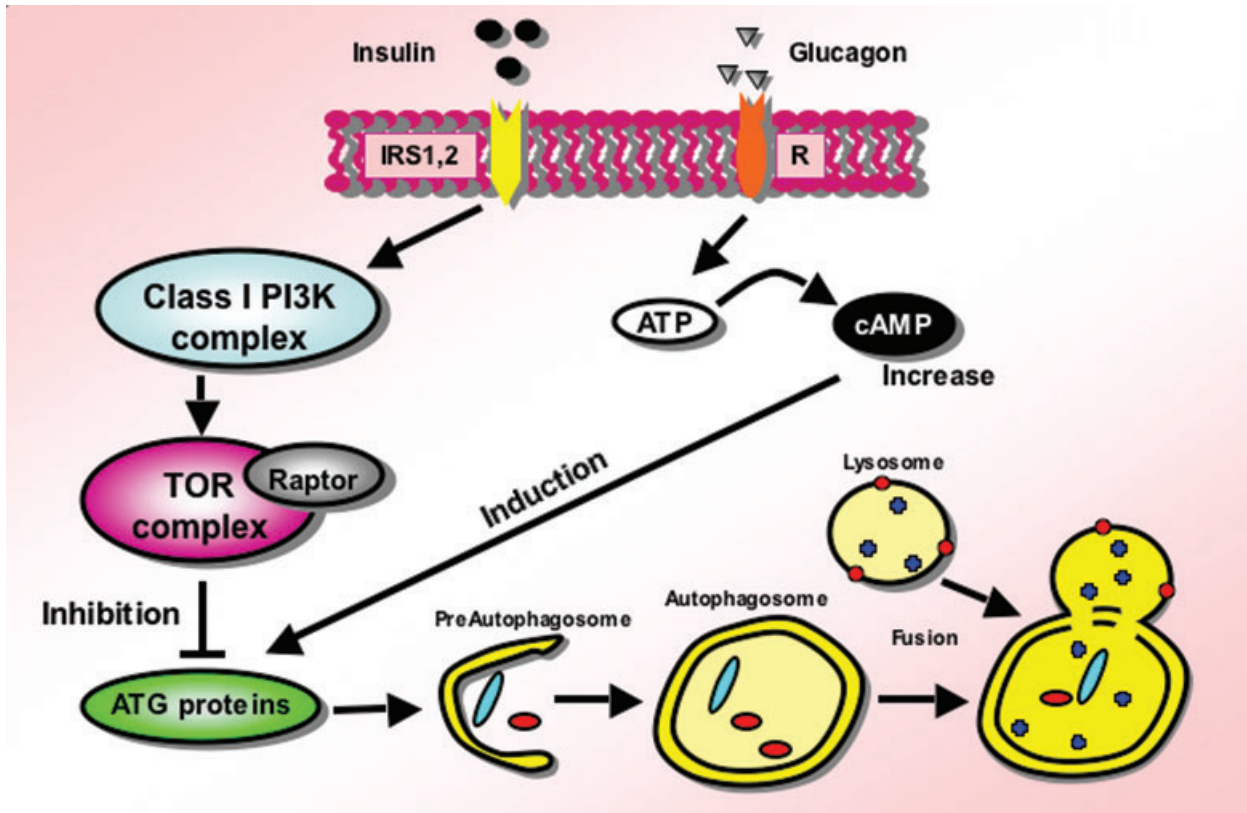
Fig. 3 Insulin signalling in autophagy. Insulin binds receptors (IRS1,2) and actives TOR complex, which dissociates from Raptor inhibiting ATG proteins. Instead, glucagon promotes autophagy by increasing of cAMP.

for the development of cardiovascular diseases, it has been suggested that adipose tissue, which is distributed in the abdominal viscera, carries a greater risk for cardiovascular disorders than subcutaneous adipose tissue. In fact, women have more subcutaneous fat, whereas men have more visceral fat. Therefore, obesity-related metabolic disorders are much lower in premenopausal women than in men.

Peripheral metabolic signals like leptin and insulin are involved in the food intake, body weight, body fat distribution and cardiovascular disease. Key areas in the brain, including the hypothalamus, integrates these peripheral adiposity signals to maintain overall adiposity levels, and these brain regions are directly influenced by sex hormones [119]. A last point concerns type 2 diabetes. Type 2 diabetes has to be considered as a gender-associated disease: sex differences play in fact a key role in the onset as well as in the progression of the disease and a higher mortality for cardiovascular diseases is detected in diabetic women with respect to men [120]. Altogether these studies validate the hypothesis of a connection between gender differences and cardiovascular diseases. However, further research to point out appropriate animal models for gender-biased studies on this issue appears as mandatory [121].

Autophagy and cell sex differences

The first direct evidence establishing the relationship between autophagy and morphological changes in androgen-dependent organs is shown by Coto-Montes *et al.* [122]. The variations in androgen levels have been shown to be the most important factor for the development of autophagy, and this information could be very useful for gaining knowledge in the field. However, the literature on this argument, *i.e.* at cellular level, is still limited. It has been suggested that 17β-estradiol could influence autophagy [123], whereas Beclin-1 seems able to down-regulate oestrogenic signalling and growth response, suggesting an important interaction between the two systems [124]. Other studies have shown that endocrine regulation could influence autophagy [125]. One example of hormone-dependent autophagic regulation is shown in hepatocytes. In fact, under low concentrations of amino acids, insulin stimulates mTOR signalling and simultaneously inhibits autophagy, whereas glucagon has the opposite effects, *i.e.* it inhibits signalling and stimulates autophagy (Fig. 3) [126]. Some animal models also provided some useful novel insight in this respect. One example can be represented by the Syrian hamster Harderian gland (HG, a gland found within the eye's orbit in several vertebrates). Different degrees of autophagy depending on sex and probably controlled by
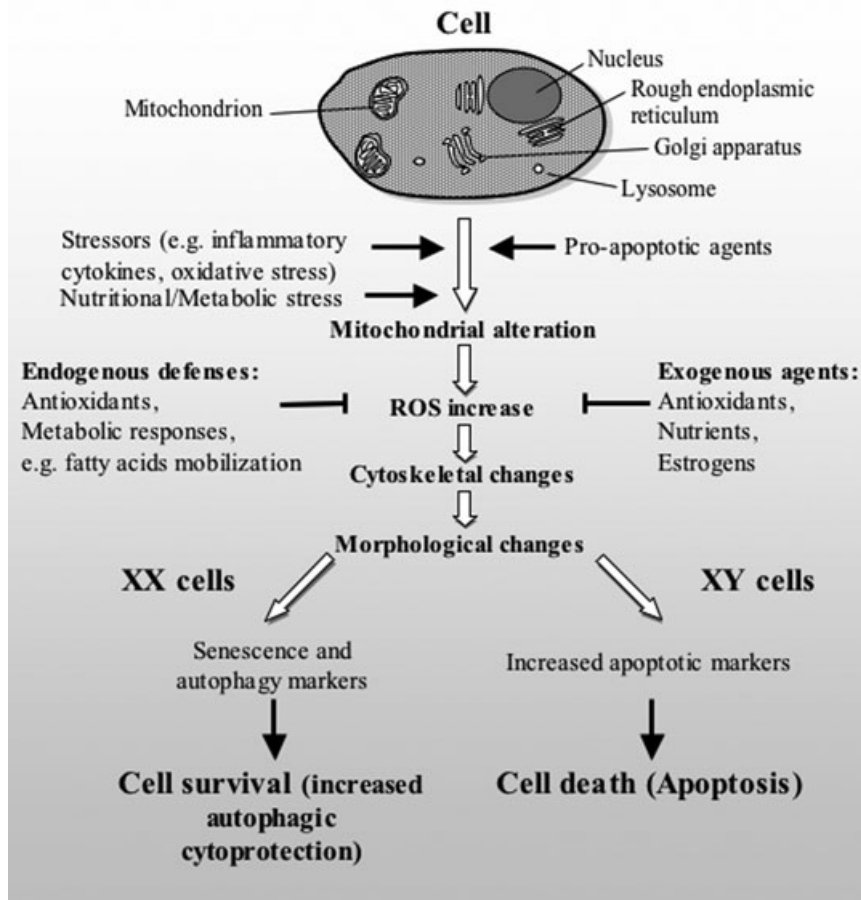
Fig. 4 Similar stressors induce a different fate in XX and XY cells. In the scheme, several actors contributing to determine cell fate are suggested. Notably, under a similar stress, *e.g.* oxidative, cells from females survive better. This could be attributed to a powered autophagic adaptive response to stressors as well as to microenvironmental alterations.

the redox-sensitive transcription factors NF-κB and p53 have been detected. Vega-Naredo *et al.* proposed a physiological significance for these phenomena: male HGs develop a survival autophagy, whereas in female HGs, autophagy culminates in a detachment-derived cell death that plays a central role in its secretory activity, leading to a massive glandular secretion [127].

Some further insights at cellular level derived from the analyses of mechanisms instructing the autophagic pathways. A paradigmatic example is represented by the insulin signalling. In fact, autophagy is regulated by several signal-transduction pathways. The most important is the insulin-growth factor-amino acid-mTOR pathway: its inhibition activates autophagy in all eukaryotes [128]. In particular, insulin hormone seems involved in autophagic pathway of a wide range of human diseases [129]. For instance, a report in the *Journal of Cell Biology* by Yamamoto *et al.* investigated the regulation of autophagy of mutant polyglutamine containing huntingtin proteins, the gene product of the disease-causing Huntington's disease gene (see above). These authors suggest that autophagy-mediated clearance of huntingtin aggregates may be triggered by the insulin-signalling pathway

[130]. Another example of autophagy regulated by hormones is represented by a study showing that 2-ME, an antitumoral compound, elicits autophagy in a c-Jun N-terminal Kinase (JNK)-dependent manner in Ewing sarcoma and osteosarcoma cells. The same authors also found that the enhanced activation of JNK by 2-ME is partially regulated by p53, highlighting the relationship of JNK and autophagy to p53 signalling pathway [131]. 2-ME has also been demonstrated to induce autophagy in MCF-7 tumour cells, helping to inhibit cell proliferation [132].

Some recent studies in isolated cells have provided some further important information as concerns hormone receptor expression and gender-dependent autophagy. Some results came from the study of VSMC (vascular smooth muscle cells) obtained from male and female rats. It was found that basal FVSMC (female-derived VSMC) express less ERα and ERβ than MVSMC (male-derived VSMC) whereas the AR level did not differ between sexes. More importantly, following induction of a stress (*e.g.* oxidative stress), the level of both AR and ERs remains unchanged in MVSMC, whereas in female-derived cells both ERs where found altered: ERβ significantly increased whilst ERα decreased [133].

Therefore, VSMC present intrinsic differences with respect to their redox state and their capability of reacting to oxidative stress and survive, being FVSMC more resistant. This can result in a higher anoikis resistance (a resistance to detachment-induced apoptosis that is called 'anoikis') of FVSMC with respect to MVSMC that can be due to their cytoskeleton-dependent adhesion features and to a higher propensity to undergo autophagy and survival in unfavourable environmental conditions. Altogether these data, obtained with contractile smooth muscle cells, could provide useful clues in the comprehension of the gender/sex differences actually detected in the pathogenesis and outcome of some diseases, including cardiovascular diseases [133].

Accordingly, on the basis of a study using isolated male and female hearts from tumour necrosis factor receptors knockout mice under acute ischemia/reperfusion injury characterized by oxidative stress, it has been suggested that ERβ mediates cardioprotection and that sex differences in the mechanisms of this tumour necrosis factor receptor2-mediated cardioprotection occur by increasing signal transducer and activator of transcription 3 (STAT3) in females and by decreasing JNK in males [134]. Because activation of STAT3 has been associated with autophagic processes [135] and it has been suggested that JNK phosphorylates Bcl-2 triggering its release from Beclin 1 in response to various stimuli [136], the existence of a different autophagic machinery between male and female cells cannot be ruled out.

Further important results have also been reported in a recent work dealing with a different cell type, *i.e.* neuronal cells [85] exposed to nutritional stress (starvation): nutrient deprivation decreases mitochondrial respiration, increases autophagosome formation and produces cell death more profoundly in neurons from males *versus* females. These authors also analyse the mechanisms of this disparity. Either Atg7 knockdown using small interfering RNA or L-carnitine, essential for transport of fatty acids into mitochondria, are more effective in neurons from males *versus* females. Tolerance to nutrient deprivation in neurons from females is associated with a marked increase in triglyceride and free fatty acid content and a cytosolic phospholipase A2-dependent increase in formation of lipid droplets. In few words, according to the results reported above, neurons from males more readily undergo autophagy and die, whereas neurons from females mobilize fatty acids, accumulate triglycerides, form lipid droplets and survive longer (Fig. 4). However, importantly, the same authors did not observe similar findings in a different cell type, *i.e.* fibroblasts. They thus suggest that autophagic response could be both sex- and tissue dependent [85].

Concluding remarks

Degradation of cytosolic proteins in lysosomes *via* autophagy has turned out to exert numerous, partly unexpected, roles in health and disease. Autophagy has been shown to contribute to innate and adaptive immunity and longevity, to the prevention of cancer and neurodegeneration. In the heart, autophagy is important for the turnover of organelles at low basal levels under normal conditions and is up-regulated in response to stresses such as ischemia/reperfusion and in cardiovascular diseases. Elucidation of the role of autophagy in mediating either cell survival or cell death, and how autophagy can be manipulated [137] would have a significant impact on the treatment of a number of human diseases. Because the study of cellular and molecular mechanisms underlying gender differences in cancer, neurodegenerative, immune and cardiovascular diseases recently gained the attention of several research groups working on gender disparity in these diseases, the analyses on the possible implication of autophagy as pathogenetic mechanism and drug target appears as mandatory and must certainly be improved in a near future. Furthermore, the advancement of our knowledge on the relationships between autophagy and hormones but, also between cell sex and autophagic cytoprotection/injury could provide important information to improve the efficacy of specific-therapies, including gender-specific therapies. In fact, the different physiology of women relative to men determines a different sensitivity and predisposition to disease resulting in a various response to therapeutic treatments (Table 1). Thus, the study of autophagic pathways and gender differences appears pivotal in the development of new and more appropriate therapeutic strategies.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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