

IRON METABOLISM AND AUTOPHAGY: A POORLY EXPLORED RELATIONSHIP THAT HAS IMPORTANT CONSEQUENCES FOR HEALTH AND DISEASE

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ABSTRACT

Autophagy is an important cell survival pathway which is up-regulated under stress conditions.¹⁾ It is a well regulated catabolic process and enables the cell to recycle its constituents and organelles for re-use.¹⁾ Autophagy has been implicated to play an important role in a variety of disorders such as cancer and protein aggregatory neurodegenerative diseases *e.g.*, Alzheimer's disease, Parkinson's disease and Huntington's disease.²⁾ Iron is a critical metal required for normal cellular functioning.³⁾ A very tightly regulated balance of iron levels is required for the normal physiological functioning of the cell.³⁾ Both an excess and deficiency of iron can lead to cellular stress, and thereby, alters the autophagic status within the cell. Thus, it is important to completely understand how iron can affect the autophagic pathway and its potential implications under physiological as well as pathological conditions.

Key Words: Autophagy, Iron metabolism, Chelators

AUTOPHAGY

Autophagy is essentially a pro-survival, catabolic process in response to stress stimuli.¹⁾ It is a recycling mechanism by which cells digest damaged or effete constituents and/or organelles ("cargo") and recycle the nutrients (*e.g.*, metals, lipids, amino acids *etc.*) back to the cell for essential processes.¹⁾ It is a well orchestrated system that starts with the formation of a crescent-shaped structure, known as a phagophore, around the damaged cargo¹⁾ (Fig. 1). This is followed by the recruitment of lipidated LC3-II protein on to the membrane, which then leads to formation of a double membrane-bound organelle, known as the autophagosome, that encapsulates the cargo.¹⁾ These autophagosomes then fuse with lysosomes to form autolysosomes¹⁾ (Fig. 1). The acidic hydrolases in the lysosomes digest the cargo and facilitate recycling of nutrients back to the cytoplasm for re-utilization.¹⁾

Autophagy plays a role in cellular detoxification and helps in clearing damaged cellular constituents.¹⁾ De-regulation of autophagy can lead to accumulation of damaged and toxic molecules, which can result in pathological conditions such as the neurodegenerative disorders: Alzheimer's, Parkinson's and Huntington's disease.²⁾ Moreover, autophagy is also known to play significant roles in tumor progression and metastasis.^{4, 5)} Autophagy has been shown to have both pro- as well as anti-oncogenic effects, depending on tumor stage and its microenvironment.⁴⁾ Some

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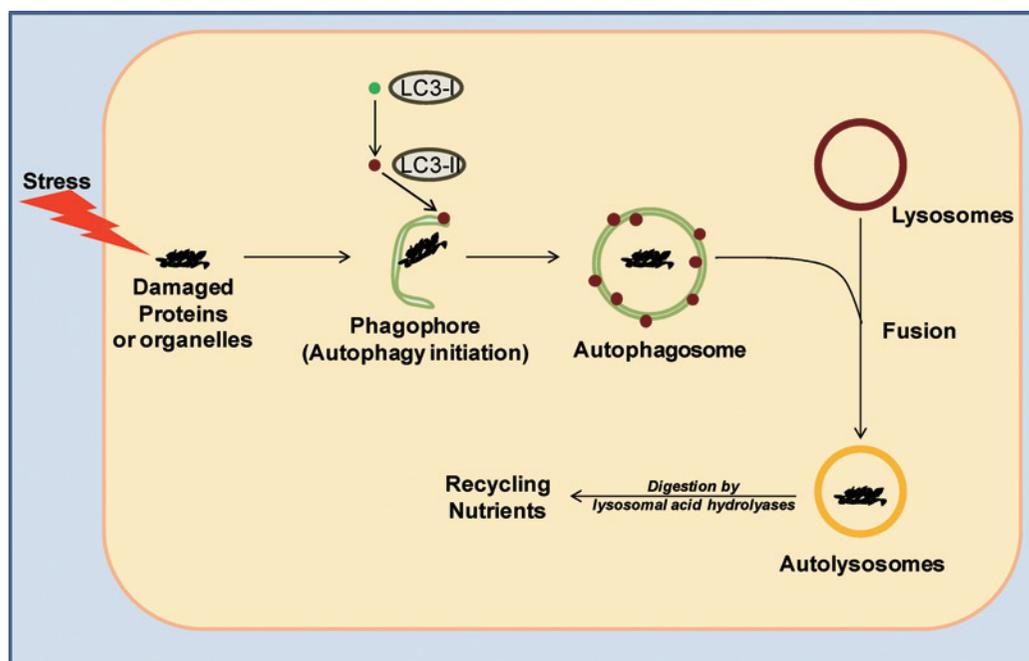


Fig. 1 The autophagic pathway: When cells experience stress it leads to an increase in damaged organelles and cellular constituents (“cargo”). This event triggers autophagic initiation and results in the formation of a crescent-shaped structure, the phagophore, around the damaged cargo. This is followed by lipidation of LC3-I to form LC3-II, which is then recruited onto the phagophore membrane. It is followed by membrane elongation and formation of double membranous organelle, known as autophagosome, around the damaged cargo. Notably, the autophagosomes are a hallmark of autophagy. The autophagosomes then fuse with lysosomes to form autolysosomes, where lysosomal acid hydrolases digest the cargo and recycle nutrients back into the cytoplasm.

studies have demonstrated the involvement of autophagy in metastasis *via* its protective role in anoikis (*i.e.*, loss-of-anchorage-dependent cell death) and cell dormancy, which are characteristics of metastatic cells.⁶⁻⁸⁾

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Iron is a requisite metal in almost all biological systems.³⁾ It is required for numerous critical processes such as DNA synthesis, heme and iron-sulfur cluster synthesis *etc.*³⁾ It also plays an important role in the active sites of various enzymes such as cytochrome *c*, aconitase, and ribonucleotide reductase.³⁾ Hence, iron is an essential bio-metal required for normal physiological functioning of the cell. However, the levels of iron in the cell need to be tightly balanced, as an excess of iron can have damaging effects due to the generation of iron-catalyzed reactive oxygen species (ROS).⁹⁾ In fact, iron can participate in the Fenton and Haber-Weiss reactions,⁹⁾ leading to ROS generation, and thus, oxidative stress. Therefore, either deprivation of iron or excessive iron levels in the cell can lead to stress and this can result in up-regulation of stress-induced autophagy. In fact, Khan *et al.* have shown that the exogenous application of iron oxide nanoparticles to cells in culture can lead to induction of autophagy due to ROS generation.¹⁰⁾

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As lysosomes are involved in autophagic turnover of organelles and long-lived proteins (many of which contain iron), they become enriched in low molecular weight iron.¹¹⁾ This increases the susceptibility of lysosomes towards oxidative stress *via* the Fenton and Haber-Weiss reactions.¹¹⁾ Moreover, the lysosomal vacuoles engaged in autophagic degradation of iron-rich proteins or organelles become more prone to oxidative stress than those in the resting state.¹¹⁾ The cytosol contains a number of iron-binding proteins (such as HSP70 and ferritin) and autophagocytosis of these proteins can potentially sequester redox-active iron in lysosomes and decrease lysosomal vulnerability to oxidative stress.^{12, 13)} Cells rich in these proteins have been demonstrated to be much more resistant to oxidative stress.¹⁴⁾ Thus, autophagy plays a critical role in balancing the redox-status of the cell *via* its ability to increase lysosomal iron levels as well as by autophagocytosis of iron-binding proteins.

Autophagy is also known to play an important role in maintaining physiological iron balance in the cell, through its role in the degradation of the iron-storage protein, ferritin.^{13, 15-23)} Ferritin is known to be involved in removal of excess iron from the cytoplasm and its storage in a non-redox active form.²⁴⁾ Indeed, ferritin has been shown to be degraded in cells by lysosomal acid hydrolases¹⁶⁾ and it is known that ferritin enters lysosomes through the autophagic pathway.¹⁵⁾ Thus, autophagy plays a critical role in the mechanism *via* which iron homeostasis is maintained in the cell for normal physiological functioning.

EFFECT OF IRON CHELATORS ON AUTOPHAGY

Currently, very little is known regarding the effect of the iron chelators on autophagy. This is important to understand considering the use of these agents for the treatment of iron overload disease, but also potentially other conditions, including cancer.²⁵⁾ Autophagy is usually characterized experimentally by measurement of autophagosomes.²⁶⁾ This can be done either directly by microscopic visualization, or indirectly by measuring the levels of lipidated LC3-II protein, which is a classical marker for autophagosomes.²⁶⁾ As autophagy is a dynamic process, the levels of autophagosomes could increase either due to elevated autophagic initiation or due to decreased lysosome-mediated degradation of autophagosomes.²⁶⁾ To determine which part of autophagy pathway (*i.e.*, initiation or degradation) is affected, it is important to utilize late-stage autophagic inhibitors, such as bafilomycin A1 or chloroquine, in the experimental protocol.²⁶⁾

A recent investigation from our laboratory has demonstrated that the chelator, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT), which forms redox-active iron and copper complexes, increases autophagosomes and LC3-II levels in pancreatic cancer cells.²⁷⁾ The effect of Dp44mT on autophagy was compared with the classical iron chelator, desferrioxamine (DFO). Although, DFO also led to a significant increase in LC3-II levels, it was markedly less effective relative to Dp44mT.²⁷⁾ Furthermore, the role of redox activity of Dp44mT was assessed by its ability to induce LC3-II. The metal complexes of Dp44mT were able to induce LC3-II levels, while the DFO metal complexes did not, as they are redox-inactive.²⁷⁾ Moreover, the anti-oxidant, N-acetylcysteine, was able to suppress the Dp44mT-mediated increase in LC3-II levels.²⁷⁾ These results indicated that ROS generation by Dp44mT-metal complexes plays a more significant role in autophagic induction than iron chelation alone.²⁷⁾

Using the late-stage autophagy inhibitor, bafilomycin A1,²⁶⁾ we demonstrated that Dp44mT increases initiation of the autophagic pathway.²⁷⁾ However, over-expression of the metastasis suppressor, N-myc downstream regulated gene-1 (NDRG1),^{28, 29)} which is also known to be up-regulated by iron chelators including, Dp44mT,³⁰⁾ resulted in suppression of Dp44mT-mediated autophagic initiation.²⁷⁾ It was concluded that Dp44mT initially increases activation of the

autophagic initiation machinery, which is then subsequently suppressed by NDRG1 up-regulation when cells were exposed to this chelator for prolonged periods of time.²⁷⁾

Interestingly, a very recent study from our laboratory has compared the autophagic-induction efficacy of Dp44mT relative to DFO in breast cancer cells.³¹⁾ It was shown that both Dp44mT and DFO led to an increase in autophagosome number and LC3-II levels.³¹⁾ While Dp44mT was able to induce the autophagic-initiation pathway, it also led to permeabilization of the lysosomal membrane, and thus, rendered tumor cells unable to complete autophagy.³¹⁾ In contrast, DFO was unable to initiate autophagy and mainly affected the lysosomal-mediated degradation phase of autophagy.³¹⁾

In another study, Wu *et al.* demonstrated that DFO induced autophagy and exerted its neuro-protective effects in the rotenone-treated SH-SY5Y cell model of Parkinson's disease.³²⁾ The authors further showed that DFO leads to the induction of autophagy *via* a hypoxia inducible factor-1 α (HIF-1 α)-dependent pathway.³²⁾ This latter investigation indicates an important role of autophagy in protein aggregatory neurodegenerative disorders such as Parkinson's disease. The difference in the ability of DFO to induce autophagy in these neuron-like cell models³²⁾ relative to breast cancer cells (Gutierrez *et al.*, unpublished data), is potentially due to the different cell-types examined.

As discussed above, ferritin is primarily known to be degraded in the cell *via* autophagy.^{15, 16)} Interestingly, Domenico *et al.* have shown that different iron chelators can specifically determine the pathway *via* which ferritin is degraded in cells.³³⁾ These authors demonstrated that poorly permeant DFO led to lysosome-mediated degradation of ferritin *via* autophagy.³³⁾ On the other hand, the membrane-permeant iron chelators, Desferasirox (DFX) and Deferiprone (DP), diverted ferritin degradation towards the proteasomal pathway.³³⁾ In contrast, another recent study has shown that both DFO and DFX lead to increased autophagy by suppression of the mammalian target of rapamycin (mTOR) signaling pathway.³⁴⁾ Again, the observed differences in the effect of DFX on the autophagic pathway may be cell-type dependent.

Critically, examining the studies above,^{33, 34)} it is notable that both investigations only visualized LC3-II-containing autophagosomes as a marker of autophagy. Considering that autophagy is a dynamic pathway,²⁶⁾ the observed increase in autophagosomes could be either due to increased autophagic initiation or because of suppression of the lysosome-mediated autophagic degradation step.²⁶⁾ Hence, in order to ascertain the reason for the observed increase in autophagosome number,^{33, 34)} it is imperative to use late-stage autophagy inhibitors,²⁶⁾ such as bafilomycin A1 or chloroquine, in combination with iron chelators. Thus, more detailed and better controlled studies will be required to determine the effect of different iron chelators on autophagy.

FUTURE PROSPECTIVES

Although, iron and autophagy play a crucial role under both physiological as well as pathological conditions, little work has been done to understand the intricate relationship between this important biological metal and how it influences the autophagic pathway and *vice versa*. More detailed investigations are required to understand the mechanisms *via* which de-regulation of iron metabolism can affect autophagy.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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