Autophagy, or “self eating,” refers to a conserved cellular process for the turnover of organelles and proteins that occurs in all eukaryotic cells.1 It is activated as an adaptive response to environmental stress (eg, nutrient deprivation, hypoxia, oxidative stress, exposure to xenobiotics) to promote cell survival through the recycling of precursors (amino acids, free fatty acids, nucleotides) derived from the degradation of endogenous cellular components. Typical of autophagy is the formation of double-membrane structures, called phagophores, that engulf intracellular material such as protein aggregates, lipid droplets, and complete organelles for degradation (Figure 1). The phagophore expands and, on completion, forms an autophagosome, which then fuses with lysosomes, thereby generating an autophagolysosome.2 Incorporation of the outer autophagosomal membrane in the lysosomal membrane allows the degradation of the remaining inner single membrane and the cytoplasmic content of the autophagosome by lysosomal hydrolases. The molecular machinery and the signaling cascades that regulate autophagy are very complex and beyond the scope of this review, but they are nicely reviewed elsewhere.3,4

Autophagy generally acts as a housekeeping mechanism, and it is crucially involved in the maintenance of normal cellular homeostasis. When stimulated by cellular stress conditions, autophagy functions as a self-cannibalization pathway that promotes cell survival in an unfavorable environment. Preclinical studies have demonstrated that autophagy is associated with cancer, neurodegenerative disorders (eg, Alzheimer, Parkinson, and Huntington diseases), embryogenesis, aging, and immunity, and also with cardiovascular disease, including ischemia-reperfusion injury of the heart, cardiomyopathy, and atherosclerosis.5–9 Despite tremendous recent advances in the field, the functional significance of autophagy in human disease remains incompletely understood and potentially involves both adaptive and mal-adaptive outcomes. Nonetheless, a growing body of evidence suggests that manipulation of the signaling pathways regulating autophagy provides novel therapeutic strategies in the prevention or treatment of human disease, as discussed for atherosclerosis in more detail below.

Autophagy in Atherosclerosis

Because of technical limitations, in particular the lack of adequate marker proteins, autophagy in atherosclerotic plaques has not yet been analyzed in full detail. As a consequence, the role of autophagy in atherosclerosis is unclear and currently under intense investigation. However, transmission electron microscopy of smooth muscle cells (SMCs) in the fibrous cap of advanced plaques revealed ultrastructural features of autophagy, such as vacuolization and formation of myelin figures.7 The latter structures, composed of phospholipids and membrane fragments, refer to autophagic degradation of membranous cellular components. Moreover, Western blot analysis of human carotid plaques showed processing of microtubule-associated protein 1 light chain 3 (LC3) into the autophagosome-specific isoform.
LC3-II, indicating activation of autophagy. Indeed, in vitro studies identified several potential triggers for autophagy that are present in atherosclerotic plaques, such as inflammation, reactive oxygen species production, accumulation of oxidized lipoproteins, and endoplasmic reticulum stress. Also, hypoxia is a common feature of advanced human atherosclerotic plaques, caused by inadequate vascularization, that is associated with nutrient and growth factor deprivation, a well-known condition leading to induction of autophagy. In addition to autophagic vesicles, myelin figures, and LC3 processing, cytoplasmic ubiquitin inclusions have been found in SMCs of human plaques. Ten years ago, detection of such inclusions by immunohistochemistry was an attractive non-electron microscopy-based technique to detect autophagy in heart failure. New insights, however, have shown that ubiquitinated inclusions in the cytoplasm of cells seem to represent autophagy failure, rather than activation of the process, as genetic knockdown of essential autophagy genes in mice leads to similar effects. Possibly, cellular damage in atherosclerotic plaques is often so overwhelming that the autophagic machinery, even in an activated state, is no longer able to remove the damaged material in an adequate way. Consequently, interpretation of autophagy data has to be done with great caution. Many groups, including our own laboratory, have measured increased autophagosome formation or LC3 processing, as mentioned above, and interpreted these events as increased autophagic activity. However, these parameters can also reflect impaired autophagic degradation. Therefore, it is essential to measure autophagic flux, which reflects the dynamic process of autophagosome formation, lysosomal fusion, and degradation. In tissue, this can be very challenging. A very elegant method now widely accepted to measure flux is using a tandem fusion protein of LC3 with the red acid-insensitive mCherry (or red fluorescent protein) protein and the green acid–sensitive green fluorescent protein to measure formation of autolysosomes and their degradation. Green fluorescent protein fluorescence is quenched in the acidic environment of the lysosome, whereas mCherry (or red fluorescent protein) fluorescence is stable at low pH. As a consequence, detection of yellow fluorescence (overlap of green fluorescent protein and mCherry) reflects impaired flux, and the presence of red fluorescence reflects quenching of green fluorescent protein and thus ongoing degradation in the lysosome. Besides LC3, levels of other autophagy substrates can be used for measuring autophagic flux, of which the best studied is p62, an adaptor protein that links ubiquitinated proteins to LC3. Accumulation of p62 reflects impaired degradation in the autophagolysosome, whereas low p62 levels indicate active degradation. Unfortunately, no data are available on autophagic flux during atherosclerotic plaque formation and destabilization.

Beneficial Role of Autophagy in Atherosclerosis
Basal autophagy can protect plaque cells against oxidative stress by degrading damaged intracellular material, in particular polarized mitochondria. In this way, successful autophagy of the damaged components promotes cell survival. The protective role of autophagy in atherosclerosis was illustrated by in vitro findings showing that SMC death induced by low concentrations of statins is attenuated by the autophagy inducer 7-ketocholesterol. Probably, the engulfment of defective mitochondria by autophagosomes limits the release of proapoptotic proteins, such as cytochrome c, into the cytosol. Also, excess free cholesterol or exposure of SMCs to lipid peroxidation products, such as 4-hydroxynonenal, activates autophagy, thereby prolonging SMC survival. Similar to SMCs, exposure of endothelial cells in culture to oxidized lipoproteins or advanced glycation end-products induces autophagy, which is protective against endothelial cell injury. Altogether, induction of autophagy represents a vital component of a general stress response in vascular cells and could therefore be an important determinant of the stability of atherosclerotic plaques (Figure 2).

Detrimental Consequences of Autophagy in Atherosclerosis
In contrast to basal autophagy, excessive stimulation of autophagy in SMCs or endothelial cells may cause autophagic death. SMC death in turn results in plaque destabilization because of the reduced synthesis of collagen and thinning of the fibrous cap. Also, endothelial cell death may be detrimental for the structure of the plaque, as endothelial injury or death represents a primary mechanism for acute clinical events by promoting lesion thrombosis. Conversely, autophagic death of macrophages is considered a promising approach to stabilize advanced plaques. Recent evidence,
neighboring phagocytes. Moreover, severe oxidative stress
induces interleukin-6 and tumor necrosis factor-
however, indicates that phagocytosis of cells dying through
autophagy results in an inflammatory response through
flammasome activation, interleukin-1β release, and subsequent
interleukin-6 and tumor necrosis factor-α induction in
neighboring phagocytes. Moreover, severe oxidative stress
combined with autophagy leads to the formation of ceroid, a
complex of protein associated with oxidized lipids. Ceroid
deposits cannot be degraded by lysosomal hydrolyses and
might lead to preferential allocation of lysosomal enzymes to
ceroid-loaded lysosomes at the expense of active autolysosomes,
which in turn would lead to progressive inhibition of
autophagy and the induction of apoptosis. In view of plaque
stability and regression, these observations might advise
against excessive autophagy induction in plaques (Figure 2).

Drug Targeting of Autophagy for Plaque
Stabilization: For Better or for Worse?
Over the past decade, there has been a true explosion of in
vitro studies discovering new or existing drugs that have the
trend to inhibit or stimulate the autophagy machinery at a
certain level in the signaling pathway, mainly in the setting
of cancer or neurodegenerative diseases. Pharmacological
modulation of autophagy can be achieved by targeting key
players in the autophagy machinery. The classical pathway
that regulates autophagy acts through mammalian target of
rapamycin, a protein kinase that plays a key role as an
intracellular nutrient sensor and controls protein synthesis,
cell growth and metabolism. For example, rapamycin or its
derivatives (eg, everolimus) inhibit mammalian target of
rapamycin, thereby activating autophagy. Another way to
modulate autophagy is to target the mammalian target of
rapamycin–independent route, mainly regulated by inositol-
1,4,5-triphosphate levels. This can be achieved with drugs
such as lithium, sodium valproate, and carbamazepine. It
should be noted that altering autophagy using pharmacological
agents may have some additional effects, as these agents
can interfere with general processes such as glucose metab-
olism (eg, metformin, deoxyglucose) or mitochondrial respi-
ration (eg, oligomycin). Moreover, broad-spectrum phospha-
tidylinositol 3-kinase inhibitors, such as wortmannin and
3-methyladenine, which are widely used in autophagy re-
search to inhibit the process, also inhibit activation of Akt. Thus
far, there has been no specific inhibitor for autophagy.
Because inhibition of Atg4 activity severely blocks au-
tophagy and Atg4-specific substrates have recently been
developed, the design of Atg4-specific inhibitors may be a
promising approach to abrogate the process of autophagy.

Induction of Autophagy in Atherosclerosis
The first line of evidence that demonstrates the beneficial
effects of autophagy induction in atherosclerosis was ob-
tained after stent-based delivery of the rapamycin derivative
everolimus in plaques of cholesterol-fed rabbits. This treat-
ment resulted in a marked reduction of macrophages via
autophagic cell death without affecting the SMCs. However,
recent in vitro work in our laboratory has demonstrated that
pharmacological induction of autophagy in macrophages
triggers secretion of proinflammatory cytokines, such as
interleukin-6, monocyte chemoattractant protein-1, Rantes,
and tumor necrosis factor-α (W. Martinet and I. De Meyer,
unpublished data, 2011), suggesting that the autophagic
process is not immunologically silent. This cocktail of cyto-
kines has the potential to induce SMC death and exacerbate
the inflammatory infiltration of leukocytes in the plaque.
Combined treatment with antiinflammatory agents, such as
glucocorticosteroids, may help to prevent the adverse effects
of drug-induced autophagy induction, hopefully without af-
fected the ability to deplete macrophages in atherosclerotic
plaques.

Excess free cholesterol induces the activation of autophagy in
SMCs in vitro as a cellular defense mechanism, possibly
through the degradation of dysfunctional organelles, such as
mitochondria and endoplasmic reticulum. Further induction
of autophagy in these free cholesterol-loaded SMCs by
rapamycin inhibited apoptotic cell death. These findings
provide a rationale for the use of rapamycin or analogs, such
as everolimus, to stabilize advanced atherosclerotic plaques.

Inhibition of Autophagy in Atherosclerosis
At present, the list of drugs with proven autophagy inhibitory
capacity is as yet very short and limited to compounds that
affect the lysosomal compartment (eg, bafilomycin A, chloro-
quione). None of these compounds have been specifically
tested in a setting of atherosclerosis. However, several studies
have shed light on the potential detrimental consequences of
autophagy inhibition with regard to plaque stability. First,
autophagy is essential for efficient phagocytosis of dying
cells. Apoptotic cells derived from cells lacking essential
autophagy genes (Atg5 or beclin-1) fail to present the “eat-
me” signal phosphatidylserine and secrete lower levels of the
“come-and-get-me” signal lysophosphatidylcholine. This will

Figure 2. Considerations for targeting autophagy as a tool for
plaque stabilization. If autophagy is occurring under basal cir-
cumstances or is moderately stimulated, it is a prosurvival
mechanism for cells to cope with stress. In this way, damaged
or unwanted components can be easily removed, and cell death
is prevented. Cells also initiate autophagy for efficient phagocy-
tosis of dead cells. Based on these findings, autophagy contrib-
utes to plaque stability. On the other hand, if cellular stress is
overwhelming or continuous and autophagy is excessively stim-
ulated, cells can undergo an autophagic type of death. More-
over, autophagic cells secrete inflammatory cytokines. This is
unfavorable because this might accelerate plaque destabiliza-
tion. However, if autophagic cell death is induced in a macro-
phage-selective manner and inflammation is pharmacologically
counteracted, local induction of autophagy is potentially benefi-
cial for stabilizing atherosclerotic plaques. Inhibition of
autophagy is most likely detrimental, because this will accelera-
te other forms of cell death (apoptosis and necrosis) and
impair the phagocytic process.

Inhibition of Autophagy
Inhibition of autophagy might be a therapeutic approach for
plaque stabilization. The following pharmacological agents
are currently considered: rapamycin, everolimus, wortmannin,
dolastatin 10, and some inhibitors of PI3K. Rapamycin inhibits
autophagy in SMCs in vitro as a cellular defense mechanism, possibly
through the degradation of dysfunctional organelles, such as
mitochondria and endoplasmic reticulum. Further induction
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result in a marked decrease in the engulfment of apoptotic corpses, enhanced plaque necrosis, and recruitment of inflammatory cells. Thus, inhibition of autophagy may further destabilize advanced plaques, where phagocytosis of apoptotic cells is already compromised (Figure 2).

Second, when autophagy is blocked in vitro by depletion of essential autophagy genes, such as beclin-1, Atg5, or Atg10, cells will not reveal autophagic vacuoles but instead are sensitized to undergo apoptosis or necrosis, indicating that a strong interaction exists between autophagy and other forms of cell death. For example, inhibition of autophagy using 3-methyladenine in free cholesterol–treated SMCs enhances apoptosis and necrosis. Moreover, in vivo experiments confirmed that the inactivation of Atg genes can cause apoptotic cell death. Autophagy proteins can also exert proapoptotic functions by themselves. For example, Beclin-1 (Atg6), a key protein involved in autophagy, can interact with the antiapoptotic proteins Bcl-2 and Bcl-xL, and this binding is apoptotic and antiautophagic, at least under nonautophagic stimuli. However, during autophagic conditions, Beclin-1 binding to Bcl-xL is antiapoptotic.

In addition, Atg5 (when cleaved by activated calpain) can bind to Bcl-xL and induce apoptosis. These data demonstrate that the cross-talk between apoptosis and autophagy should not be neglected when designing pharmacological interventions aimed at stabilizing atherosclerotic plaques via autophagy.

**Conclusion and Future Perspectives**

Autophagy may be viewed as a "cry for survival," and such survival is the result of an adaptive response to fight against cellular stress. If this stress—such as oxidative damage in atherosclerotic plaques—is mild, autophagy is activated to survive. In this way, damaged, detrimental, and unwanted components can be easily removed. On the other hand, if cellular stress is overwhelming or continuous, the adaptive response fails and cells undergo an autophagic type of death. Nonetheless, several questions remain unanswered. For example, how long can we activate autophagy without detrimental consequences for the cell? Does autophagy have a significant impact on other cell death pathways in the plaque? Crossbreeding of mouse models for atherosclerosis (eg, apolipoprotein E or low-density lipoprotein receptor knockout animals) with autophagy-deficient mice (eg, conditional Atg5 or Atg7 knockout animals) will undoubtedly help to answer these questions and may shed more light on the potential role of autophagy in atherosclerosis. Based on the literature and promising experiments in our laboratory and those of others, it is definite that cells need autophagy to be able to survive under stressful circumstances. Therefore, controlled (moderate) induction of autophagy, but not excessive induction or inhibition of autophagy, seems to be a promising strategy for stabilization of atherosclerotic plaques. The challenge for the future in the stabilization of atherosclerotic plaques will be to turn on the protective prosurvival effects of autophagy in a selective manner, without activating unwanted death pathways or proinflammatory signaling cascades. With progress being made in tissue-specific drug targeting using nanoparticles, one potential future approach to stabilize atherosclerotic plaques would be to develop macrophage-specific autophagy inducers combined with anti-inflammatory drugs. However, stimulation of autophagy can only be beneficial if autophagic flux is not impaired, because this condition could lead to lysosomal leakage or ejection of autophagosomes, as well as the induction of cell death. Furthermore, evidence is emerging for a role of histone deacetylases in both autophagy and atherosclerosis. For example, histone deacetylase-1 inhibition promotes autophagy, whereas class I histone deacetylase inhibition decreases neointima formation in a murine model of vascular injury. Because of their broad spectrum of effects and limited side effects, histone deacetylases are promising therapeutic targets for the treatment of atherosclerosis, possibly through modulation of autophagy.

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None.

**References**


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