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Autophagic Function in Atherothrombus Stabilization and Regression

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Atherosclerotic plaque formation is not irrevocable, and it as accepted that atherosclerosis could be regressed by statin treatment in clinic and experimental reports. In getting aging society, metabolic disorder become a major concern and also main cause leading cardio-and neuro-vascular disease such as atherosclerosis, stroke, and even cognitive decline. This is focusing on autophagic regression of atherosclerotic plaques. Some evidences indicate that autophagy occurs in atherosclerosis. Both in experimentally and clinically, this chapter shows that progressive plaque formation mechanism and regression under autophagic flow. On therapeutic, autophagy may be important to reveal the mechanism regressing atherosclerotic plaque.

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Key Words Autophagy, Atherosclerosis, Atherothrombus.

Atherosclerosis

Cardio and cerebrovascular disease are leading causes of mortality worldwide. Mainly, they are caused by atherosclerosis, which is a chronic inflammatory disease of blood vessels. Cellular LDL uptake is well-regulated. Their feedback mechanisms systemically limit excessive uptake and lipid overload in cells.[1] In contrast, oxidized LDL (oxLDL) mostly bypasses the feedback system, and results in intracellular lipid accumulation as foam cells present in atherosclerotic plaques.[2] The role of macrophages is critical in the development of atherosclerosis. Macrophages infiltrate to the arterial intima in response to oxLDL in the vessel. Macrophages engulf various lipids containing oxLDL and show a changed phenotype in comparison to lipid-laden foam cells. Spontaneously, they progress to a pro-inflammatory state. This is an early event in forming atherosclerotic lesion plaques.

The early events of atherosclerosis induce additional immune cell infiltration and a progressive dysfunction to initiate a cell death pathway.[2] When atherosclerotic lesions develop, apoptotic as well as necrotic cell death occur. Cell debris and cholesterol form a necrotic core in the lesion covered by a fibrous cap of variable thickness.[3] Atherosclerosis forms under chronic exposure to cellular stressors, which promotes accumulated lipid degrading cascades and consequently dysfunction. It has been revealed that macrophage autophagy is linked to lipid metabolism.[4] In atherosclerotic plaques, there is intracellular accumulation containing LDL as well as damaged tissue and misfolded/aggregated proteins.

It is apparent that inflammatory factors and inflammatory reactions play critical roles in atherothrombotic disease. This review tried to focus on the autophagic pathway and dys-regulated autophagy among contributors to athero-thrombosis.

Autophagy

In atherosclerotic plaques, there is intracellular accumulation containing LDL as well as damaged tissue and misfolded/aggregated proteins. Biologically, these extra-accumulating materials are dealt with via autophagy. Through the use of adapter proteins, the cells undergo autophagy.

Autophagy literally means "to eat oneself" and originated in Greek. It is an evolutionary conserved mechanism that is a catabolic process to degrade cytoplasmic contents such as cellular proteins and organelles through lysosomes for recycling and use in downstream metabolism.[5]

The process involves selective events rather than random bulk cleavage.[6] The selective autophagy can be described as: mitophagy, handling mitochondria; pexophagy, charging on peroxisomes; lipophagy, dealing with lipids; aggrephagy, taking care of aggregated proteins; and xenophagy, treating microorganisms. Among them, lipophagy is the initiating event by autophagy mediating a cholesterol efflux.[7] It has slowly become clear that an atherosclerotic macrophage can induce and degrade cargo lipids by selective autophagy. In the case of the chaperone protein p62/SQSTM1 in chaperone-mediated autophagy (CMA),

p62/SQSTM1 can hold and transfer polyubiquitinated cargo to autophagosomes for degradation. This machinery performs degrading events through the ubiquitin-binding domain (UBD) and the LC3-interacting region (LIR).[8] The dysregulation of this autophagic pathway results from the markedly elevated p62/SQSTM1 protein levels in macrophages of the atherosclerotic plaque.[9]

There are several types of autophagy according to the method of delivery of the cargo to lysosomes: macroautophagy, microautophgy, and chaperone-mediated autophagy (CMA).[10] Macroautophagy is the predominant mechanism among these three types. Macroautophagy starts with the formation of double-membrane vesicles, autophagosomes. Autophagosomes fuse with lysosomes and finally progress to autolysosomes. Physiological stress conditions such as starvation upregulates autophagy. Identified genes and molecules involved include around 30 genes and they are called autophagy-related genes (ATGs) required for autophagic pathways.[11]

Microautophagy raises the possibility of direct cytoplasmic engulfment by the lysosome in mammals or the vacuole in plant and fungi.[12] In macroautophagy, a double- or multi-membrane-surrounded autophagosome forms, which fuses with lysosomes in a non-specific way for degradation.[12] In contrast to in macroautophagy, in microautophagy the lysosomal/vacuolar membrane is randomly engulfed and differentiates into the autophagic tube enclosing the cytosolic portion.[13]

Another case of autophagy is chaperone-mediated autophagy (CMA). CMA targets only single proteins. In CMA, proteins are identified one by one, and the identified proteins are degraded by using a cytosolic chaperone system that delivers them to the surface of the lysosomes.[14] Selectivity in CMA uses a pentapeptide amino acid sequence motif in the substrate proteins. When the substrate proteins are recognized by a cytosolic chaperone, it results in targeting of substrates to lysosomes.[15] CMA proceeds in sequential multi-steps: 1) recognition of substrate proteins, 2) binding and unfolding of substrates, 3) translocation of substrates inside the lysosomes, and 4) degradation of substrates in the lysosomal lumen through its cellular functions.[16]

Autophagic Role in Therothrombus Stabilization and Regression Mechanism

The role of autophagy in atherosclerosis to be equivocal.[17] Primarily, autophagy is recognized as a survival mechanism and not one of the cell death pathways. Successful autophagy generally contributes to cellular survival by acting anti-apoptosis and cellular recovery by supplying biomaterials. Autophagy serves as safeguards for atherosclerotic plaque cells against cellular oxidative stress by polarizing mitochondria not to release cytochrome c.[18] In this reason, autophagy of VSMCs of the fibrous cap in advanced atherosclerotic lesion is important to plaque stabilization. Autophagic death in VSMCs results from excessively stimulated autophagy, and results in plaque destabilization.[17] Autophagic death in endothelial cells affects to maintain the structure of the thrombotic plaques. Continuously and excessively stimulated autophagy can initiate autophagic VSMCs death resulting in plaque destabilization because collagen synthesis in reduced and also the fibrous plaque cap gets thinning. Of course, autophagic cell death is triggered in endothelial cells, which is detrimental role in the sustaining structure of the atherosclerotic plaque. It is an acute clinical event promoting thrombosis on the atherosclerotic lesion.

In view of stabilizing plaque on the rupture-prone lesion, induction of autophagic macrophages might be a promising strategic role in plaque which is not obstructive into lumen but prone to rupture.[19] The transition between stable and unstable is decided by the development of a large necrotic core resulting from cell death within the plaque and failure to clearing dead cells. Macrophages are key players in the transition from stable to unstable lesions.[20] Although it is obvious that atherosclerosis is an inflammation-related arterial intima disease, pharmacologic approaches have developed the way to stabilize rupture-prone atherosclerotic lesion by applying macrophage autophagic death (Fig. 1).

Under normal conditions, cholesterol uptake of modified lipoproteins is esterified in the ER and stored in cytoplasmic neutral lipid droplets in macrophages.[21] Under cholesterol-overloading conditions, cholesterols are unesterified and stacked in the ER membrane of macrophages, leading to ER stress.[21] Along with ER stress induction, activation of pattern-recognition receptors (PRRs) may induce apoptosis in macrophages.[22] Macrophages are responsible for efferocytosis in atherosclerotic plaques as the main phagocytes. At the beginning, macrophage apopto-

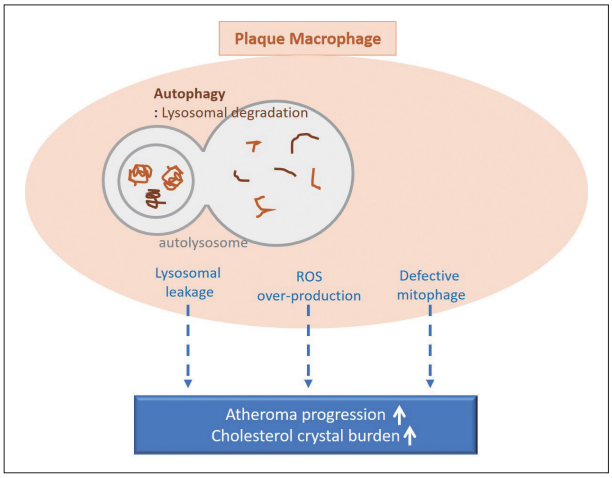

Figure 1. Autophagy in Atherosclerotic Progression. Autophagic dysfunction might invite lysosomal leakage, overproduction of reactive oxygen species (ROS), and mitophagy impairment. Sequential processes increase accumulation of cholesterol crystals. Under autophagy deficiency, this process might lead to atherosclerotic propagation.

sis couple with effective efferocytosis contributing to a reduction plaque size.[22] As atherosclerotic process, clearing apoptotic cells is getting defective and necrosis occurs. This stage allows macrophage cellular contents to release and to form a large necrotic core as a consequently.[22]

In human atherosclerotic plaques, efferocytosis is impaired and also shaded phagocytic receptors, which impedes phagocytic capacity of macrophages and involves activation of the inflammatory response.[23] Plasma levels of soluble CD36, one of scavenger receptors, are higher in the context of risk factors for the development of atherosclerosis such as diabetes.[24] The altered "eat-me" signals can also affect efferocytosis and the targets of apoptotic cells. In another study, mice laking complement factor C1q exhibited efferocytosis dysfunction and atherosclerotic plaque burden.[25]

Autophagic Role in Atherothrombus Regression

Atherosclerosis progression presents the features of impaired autophagy. Autophagy is sequential events called as autophagic flux (autophagosome formation, cargo sequestration, and autolysosomal fusion), and unfortunately hardly to assess the flux *in vivo*. Atg5 knock-out mice with ApoE-null background showed that western diet for 2 months increased the p62/SQSTM1 in the vessel with similar level of control mice whereas atherosclerotic lesion was bigger than control both in aortic root and whole aorta.[26] Using animals with experimental atherosclerosis, ApoE-null mice, recent study proposed that plaque formation expands when macrophagic autophagy is completely disrupted not partially disrupted. Partially disrupted autophagic condition induces rather macrophagy inflammation and excess IL-1beta, because cholesterol crystal of atherosclerotic plaques is potent stimuli to activate inflammsome.[26]

Cholesterol efflux is induced to balance the level of macrophage storing lipid by transferring increased cholesterol from peripheral tissues to the liver. The primary cholesterol efflux mechanism has been thought that cholesterols are hydrolyzed cholesteryl esters cytosolic hydrolases; free cholesterols are moved to the plasma membrane; finally free cholesterols are delivered to the periphery by ATP-binding cassette transporters (ABCA1 or ABCG1).[27]

Autophagic malfunction of macrophages abrogates this cholesterol efflux when macrophages are faced to hinder autophage with chemically (chloroquine) or genetically (Atg5-deficiency). Furthermore, inhibitors of lysosomal acid lipase also diminish cheolesterol efflux. These are showed that cholesterol hydrolysis as well as autophagic delivery is critical step in atherosclerotic plaque progression and regression. Although lipid-laden macrophages induce lipophagy and also trigger a counter regulatory mechanism are unclear, it is clear that lipophagy-mediated efflux play an important role in cholesterol transport *in vivo*.[4] Therefore, efficient cholesterol metabolism and efflux are considered athero-protective mechanisms against accumulated lipid-laden atherogenic condition.[28]

Prospects and Conclusions

For developing new therapeutic strategies for atherosclerosis, it is important to understand the cause of the disease pathogenesis and its progression. A number of studies have shown that cell deaths produce different patterns depending on the stage of the plaque and types of cells involved in cell death. Lipid reduction is the one of the well-known ways to eradicate macrophages from atherosclerotic plaque. Statins are generally used in myocardial infarction patients. Taken together, this review suggests the possibility that activation of autophagy could interfering with the statin-induced apoptotic pathway.

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