Introduction

Insulin resistance is a major metabolic abnormality in the great majority of patients with type 2 diabetes, and in aging people. Obesity is clearly the most common cause of insulin resistance. The etiology of insulin resistance has been well studied, and several recent studies have implicated chronic tissue inflammation as an important cause of obesity-induced insulin resistance. Furthermore, studies demonstrate that a key mechanism underlying obesity-induced inflammation is the accumulation of tissue macrophages, T cells and neutrophils in obese adipose tissue. These lines of evidence indicate that adipose tissue is an important etiologic tissue in insulin resistance and chronic inflammation. This insulin resistance in adipose tissue leads to systemic insulin resistance, including that in liver and muscle. However, inflammation is not the only mechanism of insulin resistance. Recent studies have also indicated a contribution from several sources of stress including hypoxic stress, oxidative stress, and ER stress.

Autophagy is the mechanism by which cytoplasmic components such as protein aggregates, end-products and organelles are broken down and recycled through the lysosome. For a long time, autophagy has been considered as a system for protein turnover and a mechanism for replenishing the intracellular amino acid pool during starvation. It was thought to be simply a mechanism by which a cell, when no nutrition is available in the surrounding environment, is forced to break down part of its own reserves to stay alive until the situation improves. However, it is becoming increasingly clear that a homeostatic low level of autophagy is important for Anti-Aging, regular maintenance, and disposal of intracellular proteins and organelles.

In an insulin-resistant state, various dysfunctional/damaged components are retained and can lead to cellular stresses and/or inflammation (as mentioned above); we therefore speculated that autophagy has an important role in ‘cleaning up’ these molecules. It has been well documented that insulin inhibits autophagy. However, the insulin signaling pathway is suppressed in an insulin-resistant state. Therefore it is important to resolve the issue of whether autophagy is suppressed or activated in insulin resistance. Recently, several reports about autophagy in insulin resistance, including tissue-specific knockout mice and in vitro experiments, have been published. In this review, I have focused on the connection between autophagy and insulin resistance.

Review Article

Autophagy in Insulin Resistance

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Abstract

This article reviews autophagy as a factor linked to insulin resistance. In both insulin-resistant and aging cells, numerous stresses such as oxidative stress, hypoxic stress, ER stress and inflammation are observed. These stresses cause accumulation of various dysfunctional/damaged molecules, organelles, proteins and end-products. Therefore, removal of these accumulated molecules can have clinical value in combating insulin resistance as well as aging.

Autophagy, which is strikingly induced by starvation, is an essential process by which cells break down their own components. In addition to this, a homeostatic low level of autophagy is critical to ‘clean up’ intracellular organelles and proteins. Thus, defective autophagy decreases the removal of potentially damaging molecules and increases their accumulation, which causes insulin resistance. In insulin-resistant mouse models, autophagy is suppressed in many tissues and several tissue-specific autophagy knockout mice reveal insulin resistance and glucose intolerance. These studies strongly suggest that autophagy has an important role in insulin resistance. Further study of autophagy may help identify novel anti-insulin-resistance and Anti-Aging treatments.

KEY WORDS: Autophagy, insulin resistance, aging, inflammation
Autophagy in Insulin Resistance

**Lysosome-autophagy pathway**

When intracellular proteins and organelles become damaged through aging or various stresses, this can cause damage to the cell as a whole. Cells have two major systems to remove these potentially damaging molecules: the lysosome-autophagy system, and the ubiquitin proteasome system. Of these, autophagy is the only one able to degrade large molecules.

The mechanism underlying the process of autophagy has been extensively investigated and reviewed in detail [15]. Briefly, as shown in Fig. 1, autophagy starts with an expanding membrane sac termed the phagophore, which surrounds portions of the cytoplasm including proteins and organelles, leading to the formation of a double-membrane-bound vesicle called the autophagosome. Next, the autophagosome becomes fused with a lysosome and thus changes into an autolysosome, in which the inner components are exposed to lysosomal hydrolases. During this process, large molecules in the autolysosome are broken down to the amino acid level and released into the cytosol for recycling.

Three forms of autophagy have been described, namely macroautophagy, microautophagy and chaperone-mediated autophagy [16]. In this review, I have focused on macroautophagy, a potent degradation mechanism that can turn over and eliminate cytosolic proteins and organelles in the state of insulin resistance. Autophagy induced by starvation is an essential process by which cells break down their own components. This type of autophagy is called stress-induced autophagy. Meanwhile a homeostatic low level of autophagy is important for disposal and maintenance of intracellular proteins and organelles. This basal-type autophagy is needed for cellular quality control and Anti-Aging, and is called homeostatic autophagy. Recently, homeostatic basal autophagy was reported to be the type of autophagy in which a defect caused neurodegenerative disease [17], and this has been widely observed.

**Autophagy activity in insulin resistance**

The first question is whether autophagy is activated or suppressed in an insulin resistant state. Several reports referring to the activity of autophagy in insulin target tissues have been published. In a study in db/db mice with diet-induced obesity, autophagosomal formation was observed along with an increase in β-cell number, indicating that autophagy is activated in insulin-resistant pancreatic β cells [18,19]. In contrast, hepatic autophagy was reported to be suppressed in insulin resistance, as shown by decreased LC3-II and increased p62 detection by western blotting [20,21]. In addition to this, we have reported that autophagy was also suppressed in adipose tissue from high fat-diet (HFD)-induced insulin-resistant LC3-GFP transgenic mice [22]. Furthermore, decreased autophagy has been observed in macrophages with atherosclerosis-related stimulation [23], in muscle [24] in hypothalamus [25] and in kidney [26] from HFD-induced insulin-resistant mice. Overall (Fig. 2), although the mechanism for reduced autophagy is not clear, autophagy seems to be suppressed in the insulin-resistant state. However, since the activity of autophagy changes under various conditions, we should be attempting to investigate the response of autophagy to insulin resistance in a range of mouse models, such as genetic models, or due to short or long time exposure to HFD. We should also pay attention to the degree of insulin resistance in human studies.

![Fig. 1. Autophagosome formation.](image)

The cellular events during autophagy.
Autophagy knockout models in insulin resistance

The findings presented in the previous section raise questions as to the potential role of autophagy in insulin resistance. To assess this, tissue-specific knockout mice studies were utilized (Fig. 3). The deletion of the autophagy-related gene \textit{Atg7} in pro-opio-melanocortin neurons led to increased food intake, weight gain, and fat pad weight \cite{27}. Moreover, there was a shift in adipocyte size distribution toward larger adipocytes and this correlated with impaired glucose tolerance and adipose tissue inflammation. While these findings are important for insulin resistance, additional investigations, including investigation of hypothalamic neuron subtypes, and regulation of appetite and circadian rhythm, are necessary to better understand the global effects of this deletion.

\textit{Atg7} and \textit{Atg5} knockout in hepatocytes caused accumulation of lipid droplets \cite{21} and elevation of ER stress \cite{28}. These were accompanied by decreased insulin signaling and impaired glucose tolerance. The mechanism of autophagy decline was investigated in hepatocytes, and this showed that obesity caused an increase in calcium-dependent protease calpain 2, which in turn caused decreased expression of \textit{Atg7}. Restoration of \textit{Atg7} expression by inhibition of calpain supported this observation \cite{28}. Although these data have not yet been published, very recently it has been suggested that autophagy in liver plays a role in the development of non-alcoholic steatohepatitis, non-alcoholic fatty liver disease and hepatocellular carcinoma. This is an area of considerable current interest, and we anticipate progression of this investigation in near future.

In muscle, suppression of autophagy in insulin resistance has been confirmed. However, the knockout mice used in this study \cite{24} showed normal levels of basal autophagy, but were deficient in exercise- or starvation-induced autophagy. This defect in exercise-induced autophagy attenuated the protective effect of long-term exercise against HFD-induced glucose intolerance. Although the role of a homeostatic basal level of autophagy in muscle remains unclear, sarcopenia has been suggested as one cause of insulin resistance in aging \cite{29} and basal autophagy is thought to regulate sarcopenia. Thus, it is easy to conclude that autophagy is a key regulator of sarcopenia, although further study in this field is needed.

Recently it has been well established that adipose tissue macrophages play an important role in insulin resistance. Adipose tissue macrophages accumulate in obese adipose tissue, where they can enhance local inflammation to cause the development of tissue insulin resistance. There has been no report to date regarding macrophage-specific autophagy knockout mice, nor has any bone marrow transplantation study on adipose tissue insulin resistance been reported. However, two
Autophagy in Insulin Resistance

reports have been published in atherosclerosis. Both of these showed that defective macrophage autophagy promoted plaque necrosis, macrophage apoptosis and oxidative stress in advanced atherosclerosis, suggesting that autophagy in macrophages could play a role in protection against pro-inflammatory responses in adipose tissue. Future studies are likely to reveal additional roles of macrophage autophagy in insulin resistance.

Finally, adipose tissue plays a central role for systemic insulin resistance. Singh et al. and Zhang et al. reported adipose-specific deletion of autophagy. However, the case in adipose tissue is complicated. These mice indicate that autophagy is involved in adipogenesis. When any of the genes involved in adipogenesis are depleted in adipocytes, a lean phenotype, smaller adipocytes, and protection against diet-induced obesity are usually observed. Since the adipocytes in knockout mice have not matured, these phenotypes do not accurately reflect the role of these molecules when they are depleted in the mature adipocyte. Further studies such as those in conditional knockout mice should be conducted. The study of autophagy inhibition in mature adipocytes has shown that suppression of autophagy can cause ER stress and inflammation in adipocytes, indicating that a defect in autophagy may lead to the accumulation of dysfunctional molecules which cause inflammation. Increased macrophage accumulation was observed in adipose tissue from adipose-tissue-specific autophagy knockout mice in spite of the presence of smaller adipocytes, and these animals are at high risk of early death. Taken together, these data suggest that the role of autophagy in adipocytes is important for the maintenance of cellular health.

Aging and the Future

A common characteristic of aging cells is the accumulation of damaged/dysfunctional molecules. Autophagy has been reported to decrease with aging. A gradual decrease of autophagy with aging could play an important role in the functional deterioration of aging tissue. As discussed above, in the insulin resistance state, dysfunctional molecules also accumulate. We might therefore expect to see similar phenomena in both insulin resistance and aging, although over a different time course. Therapeutic approaches to activate autophagy may therefore provide a new strategy to prevent both insulin resistance and aging.

In order to manipulate autophagy for novel potential therapeutic purposes, we need to improve the level of information about many potentially related, but as-yet poorly defined, issues, and we should also carefully consider several other types of disease, such as neurodegeneration, cancer, and immunity. With a more comprehensive understanding of autophagy, there should be potential to modulate autophagy activity for improved therapeutic approaches to both human insulin resistance and aging.

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Conflict of interest statement

The authors declare no financial or other conflicts of interest in the writing of this paper.

Fig. 4. A proposed mechanism by which defects in autophagy cause insulin resistance.
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