

Cultured Cells Exposed to Oxidative Stress

Subjects: [Nutrition & Dietetics](#) | [Psychology, Biological](#) | [Medicine, Research & Experimental](#)

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In 1972, Brunk and Ericsson found that significant amounts of lysosomal acid phosphatases leak through the ultrastructurally intact lysosomal membrane in cultured glioma cells. Subsequently, Brunk and his colleagues established the concept of lysosomal membrane permeabilization (LMP) in a series of works using cultured cells which were exposed to artificial oxidative stress.

calpain-cathepsin hypothesis

carbonylation

electron microscopy

1. Introduction

Lysosomes digest, recycle, and dispose of aged/damaged organelles, macromolecules, or proteins within the cell in both physiological and pathological conditions. A limiting membrane seals off their hydrolytic enzymes and prevents leakage into the cytoplasm in order to not damage the cell. In the early 1950s, Christian de Duve and his colleagues demonstrated that the lysosomal acid phosphatase is not detectable within the fresh cell homogenates, but can be detected in large quantities after cell fractionation through the differential centrifugation method. Furthermore, with the aid of an electron microscope, they observed numerous membrane-bound, tiny organelles in the cell fraction. These findings led them to speculate that acid phosphatase must be sealed in a sac, but it may break and release its content in response to severe insults. This phantom enzyme provided de Duve a clue to the serendipitous discovery of the 'lysosome' [\[1\]\[2\]\[3\]](#).

In the late 1960s, considerable interest was focused on the implication of lysosomal involvement in cell degeneration and necrosis. In 1966, de Duve and Wattiaux reported the concept that lethal cell injury occurs in pathological states by the release of hydrolytic enzymes from damaged lysosomes. They suggested that it might be an early and triggering event for cell injury [\[4\]\[5\]](#). In 1972, Brunk and Ericsson found that significant amounts of lysosomal acid phosphatases leak through the ultrastructurally intact lysosomal membrane in cultured glioma cells [\[6\]](#). Subsequently, Brunk and his colleagues established the concept of lysosomal membrane permeabilization (LMP) in a series of works using cultured cells which were exposed to artificial oxidative stress [\[7\]\[8\]\[9\]\[10\]\[11\]](#). They thought that acid phosphatases or other lysosomal enzymes may leak through the ultrastructurally intact lysosomal membrane, but not from the rupture sites at the membrane. The concept of LMP was widely accepted thereafter [\[12\]](#). Therefore, for a long time, the lysosome has been imprecisely considered a sturdy organelle that does not disintegrate until the cell is already devitalized [\[13\]](#). However, in 1996, Yamashima et al. found through electron microscopy that the rupture of the lysosomal-limiting membrane associated with the extra-lysosomal release of cathepsin enzymes occurs in the programmed necrosis of hippocampal *cornu Ammonis* 1 (CA1) neurons of macaque monkeys a few days after transient global brain ischemia [\[14\]](#). This was the first report of lysosomal

rupture, because the concept of apoptosis had been prevailing in the 1990s as a mechanism of programmed cell death.

Nowadays, it is believed that a low level of cell stress causes LMP and apoptosis, whereas a high level of cell stress causes lysosomal membrane rupture and necrosis [15][16][17]. LMP was suggested to induce apoptosis by mitochondrial transmembrane potential loss or caspase activation. In contrast, lysosomal membrane rupture induces necrosis due to the extensive leakage of cathepsin enzymes [10][11][13][14][18][19][20][21][22][23]. Traditionally, necrotic cell death was considered a passive death mode, which is triggered by catastrophic events such as heat shock, ischemia, irradiation, or irreparable stress to the cell [24]. More recently, many of these 'unregulated' cell death events were found to be essentially 'regulated', and the '*calpain-cathepsin hypothesis*' was formulated to explain the mechanism of programmed neuronal necrosis in 1998 [25].

Lipid peroxidation product 4-hydroxy-2-nonenal (hydroxynonenal) is generated during deep-frying in cooking oils and/or within biomembranes by the long-standing oxidative stress. It may be either protective or damaging to the cells, depending on its concentration [26]. For example, at low concentrations, hydroxynonenal is involved in the control of signal transduction, gene expression, cell proliferation, differentiation, and cell cycle regulation. At high concentrations, however, hydroxynonenal forms adducts with proteins, nucleic acids and membrane lipids, which leads to long-term cell disorder and tissue damage [27][28]. Lifestyle-related diseases are defined as health disorders which are linked to the way people live their life. Representative diseases that are affected by the lifestyle are chronic diseases such as Alzheimer's disease, type 2 diabetes, nonalcoholic steatohepatitis (NASH), ischemic heart diseases, etc. Since the etiology of these lifestyle-related diseases is complex and varies among individuals, identification of the most essential causative substances is very difficult, especially if their relative effect, i.e., acute toxicity, is weak and many years are needed for the disease progression due to chronic toxicity. Deep-frying using vegetable oils is a popular culinary method used worldwide, but the high oil content is a major health concern. For example, in the United States, nearly half of potatoes produced are deep-fried and processed into chips and French fries [29]. Hydroxynonenal is recently thought to be a key factor for damaging cells of the brain, pancreas, and liver [18][23][30][31]. However, the causative substance of lifestyle-related diseases, if present, still remains unknown. Recently, Yamashima et al. reported the occurrence of lysosomal membrane rupture/permeabilization in the neurons, Langerhans islet cells, and hepatocytes of monkeys after consecutive injections of synthetic 'hydroxynonenal'. They speculated that the ω -6 polyunsaturated fatty acid (PUFA)-peroxidation product, which is contained in deep-fried foods cooked by linoleic acid-rich cooking oils, is the most suspicious causative substance of lifestyle-related diseases [18][23][30][31].

2. Cultured Cells Exposed to Oxidative Stress

The concept of LMP was first reported half a century ago in cultured cells treated with lysosomotropic detergents [6]. However, the interest in implicating lysosomes in necrotic cell death faded during the following two decades, and, concomitantly, lysosomal involvement in necrosis has been overlooked. This is presumably because the ability of caspase inhibitor Z-Val-Ala-Asp-fmk (zVAD-fmk) to inhibit cell death was considered as proof of not "necrosis" but "apoptosis". Furthermore, many researchers had failed to detect LMP by conventional electron

microscopic observation, because the lysosomal membrane looked grossly intact [32]. So, the presence of LMP was suggested initially not by conventional electron microscopy but by immunoelectron microscopic analysis. By analyzing the cytotoxicity of oxidized low-density lipoprotein to cultured macrophages, Li et al. (1998) observed extra-lysosomal release of cathepsin D using immunoelectron microscopic analysis [33]. Similarly, by exposing the cultured myocytes of a neonatal rat heart to oxidative stress using redox cycling quinone naphthazarin, Roberg and Öllinger (1998) observed both leakage of intra-lysosomal cathepsin D into the cytoplasm and dissolution of myofilaments through immunoelectron microscopic analysis [34].

By exposing the cultured hepatocarcinoma cells to the synthetic hydroxynonenal, Seike et al. recently observed lysosomal membrane rupture using conventional electron microscopic analysis [18]. The fluorescence time-lapse imaging of the HepG2 hepatocarcinoma cell lines clearly showed a rapid loss of lysosomes (being stained orange by LysoTracker) at the early phase of cell necrosis. This is the first animation which demonstrated an implication of lysosomal rupture in the occurrence of necrotic cell death [18]. The addition of hydroxynonenal in the cultured medium induced 'bursting' necrosis which was closely associated with the gradual reduction and loss of lysosomes in the early phase. Hydroxynonenal induced neither apoptotic bodies nor cell blebbings, but shrinkage of both the nucleus and the cytoplasm was observed. In contrast, the addition of an anti-cancer agent, epirubicin hydrochloride, in the medium induced apoptotic cell death with the formation of cell blebbings and apoptotic bodies, but lysosomes remained intact until the final phase, just prior to the cell death. Obviously, hydroxynonenal induced lysosome-mediated necrosis, whereas epirubicin hydrochloride induced lysosome-unrelated apoptosis.

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