Impact of Oxysterols

Subjects: Medicine, Research & Experimental

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Oxysterols are oxidized derivatives of cholesterol produced by enzymatic activity or non-enzymatic pathways (auto-oxidation). The oxidation processes lead to the synthesis of about 60 different oxysterols. Several oxysterols have physiological, pathophysiological, and pharmacological activities. The effects of oxysterols on cell death processes, especially apoptosis, autophagy, necrosis, and oxiapoptophagy (a complex mode of cell death characterized by ROS overproduction ("oxi-"), apoptosis induction, ("-apopto"), and autophagy ("-phagy")), as well as their action on cell proliferation, are reviewed here. These effects, also observed in several cancer cell lines, could potentially be useful in cancer treatment. The effects of oxysterols on cell differentiation are also described. Among them, the properties of stimulating the osteogenic differentiation of mesenchymal stem cells while inhibiting adipogenic differentiation may be useful in regenerative medicine.

Keywords: apoptosis ; autophagy ; cell death ; differentiation ; oxysterol ; Oxiapoptophagy

1. Introduction

Cholesterol (cholest-5-en-3β-ol) is a major sterol (steroidal lipid) present in mammalian cells [1]. It is an important cell membrane compound with crucial roles in cell growth and proliferation. Cholesterol is involved in membrane biogenesis and function, controlling its physical properties such as fluidity and curvature [2][3][4]. In addition to this structural role, cholesterol also has several other functions: it is a precursor to bile acids, to vitamin D, and to a variety of steroid hormones [2]; it is involved in several intracellular signal transduction processes [5]; it regulates protein function [6]; it participates in myelin formation [7]; it acts as ligands to nuclear receptors and to G protein-coupled receptors (GPCRs) [8][9]. Cholesterol is very susceptible to oxidation [10][11][12], which favors the formation of derivatives called oxysterols [2][11][13], which can be found in low concentrations in the organism (nanomolar range in the plasma of healthy subjects) [14][15].

2. Oxysterols

Oxysterols are a large family of 27-carbon oxidized derivatives of cholesterol [16]. They are endogenously produced in vivo by a variety of cells via enzymatic activity, auto-oxidation (radical processes), or both [2][13][17][18]. Several major oxysterols arise as intermediates in the pathways converting cholesterol to bile acids or steroid hormones [19][20][21]. As a result of cholesterol oxidation, polar groups (hydroxy, keto, hydroperoxy, epoxy, or carboxyl) are added to the cholesterol molecule. In addition, oxysterols are also present in the diet. Phytosterols, which are oxygenated forms of plant sterols and some cholesterol precursors, can also originate oxysterols [22][23][24][25].

Figure 1 shows several oxysterol derivatives. **Table 1** lists some oxysterols with their common names, as well as their names according to the International Union of Pure and Applied Chemistry (IUPAC).

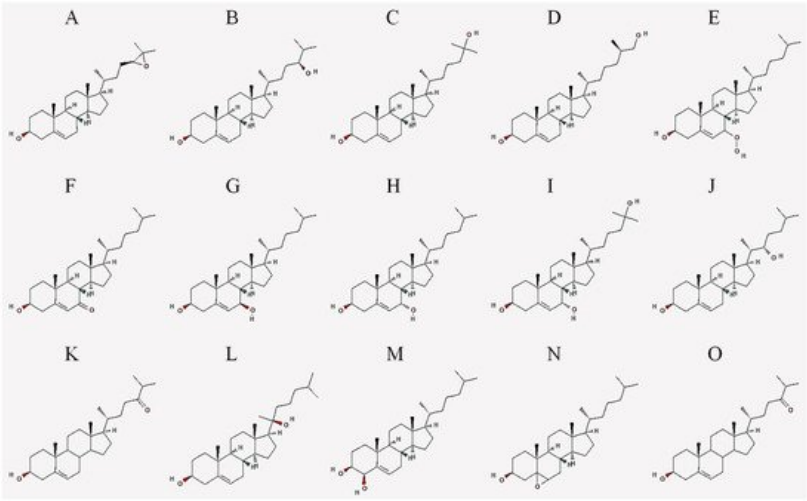

Figure 1. Oxysterol 2D molecules representations. (**A**) 24(S),25-epoxycholesterol; (**B**) 24(S)-hydroxycholesterol; (**C**) 25-hydroxycholesterol; (**D**) 27-hydroxycholesterol; (**E**) 7-hydroperoxycholesterol; (**F**) 7-ketocholesterol; (**G**) 7β-hydroxycholesterol; (**H**) 7α-hydroxycholesterol; (**I**) 7α,25-dihydroxycholesterol; (**J**) 22(S)-hydroxycholesterol; (**K**) 24-oxocholesterol; (**L**) 20(S)-hydroxycholesterol; (**M**) 4β-hydroxycholesterol; and (**N**) 5,6-epoxycholesterol; (**O**) 24-oxocholesterol. Source: PubChem; URL: https://pubchem.ncbi.nlm.nih.gov; last accessed, 5 August 2021.

Table 1. Common names of the main oxysterols and their chemical nomenclature.

Abbreviation	Common Name	IUPAC Name
24,25-EC	24(S),25-epoxycholesterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R)-4-[(2S)-3,3-dimethyloxiran-2-yl]butan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
24-HC	24(S)-hydroxycholesterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5S)-5-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
25-HC	25-hydroxycholesterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R)-6-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
27-HC	27-hydroxycholesterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,6R)-7-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
7-OOHC	7-hydroperoxycholesterol	(3S,8S,9S,10R,13R,14S,17R)-7-hydroperoxy-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
7α-HC	7α-hydroxycholesterol	(3S,7S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol
7β-HC	7β-hydroxycholesterol	(3S,4R,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,4-diol
7-KC	7-ketocholesterol	(3S,8S,9S,10R,13R,14S,17R)-3-hydroxy-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-1,2,3,4,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-7-one
7α,25-DHC	7α,25-dihydroxycholesterol	(3S,7S,8S,9S,10R,13R,14S,17R)-17-[(2R)-6-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol
7β,27-DHC	7β,27-dihydroxycholesterol	(3S,7R,8S,9S,10R,13R,14S,17R)-17-[(2R)-7-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol
22-HC	22(S)-hydroxycholesterol	(3S,8S,9S,10R,13S,14S,17R)-17-[(2S,3S)-3-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol
20-HC	20(S)-hydroxycholesterol	(3S,8S,9S,10R,13S,14S,17S)-17-[(2S)-2-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol

Abbreviation	Common Name	IUPAC Name
4β-HC	4β-hydroxycholesterol	(3S,4R,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,4-diol
7,25-DHC	7,25-dihydroxycholesterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R)-6-hydroxy-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,7-diol
5,6-EC	5,6-epoxycholesterol	(1S,2R,5S,11S,12S,15R,16R)-2,16-dimethyl-15-[(2R)-6-methylheptan-2-yl]-8-oxapentacyclo[9.7.0.02,7.07,9.012,16]octadecan-5-ol
24-OXO	24-oxocholesterol	(6R)-6-[(3S,10R,13R,17R)-3-hydroxy-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-methylheptan-3-one

Source: PubChem; URL: https://pubchem.ncbi.nlm.nih.gov; accessed on 5 August 2021.

In the enzymatic pathways, oxysterols are formed by the action of several specific enzymes. There are three groups of enzymes associated with oxysterol metabolism: oxidoreductases (e.g., cytochrome P450, cholesterol hydroxylase, hydroxysteroid dehydrogenases, and squalene epoxidase); hydrolases (e.g., cholesterol epoxide hydrolase, and cholesterol esterase); and transferases (e.g., hydroxysteroid sulfotransferases, acyl-CoA cholesterol transferase, and lecithin-cholesterol acyltransferase) [10][13]. Oxysterols generated by enzymatic processes commonly have oxidized side chains [10][26]. Interestingly, some genetic alterations of these enzymes, involved in oxysterol formation, have been associated with some types of cancer: *CYP7A1* gene polymorphism -204A > C, rs3808607 has been associated with colorectal cancer [27] while polymorphisms -204 A > C, rs3808607, and -469 T > C, rs3824260, have been associated with increased gallbladder cancer risk [28]; *CYP3A4*1B* gene polymorphism rs2740574 A > G has been associated with an increase in prostate cancer among African populations [29]; decreased gene expression of 11β-HSD1 and overexpression of 11β-HSD2 have been described in breast cancer [30][31].

Endogenous oxysterols are commonly produced by non-enzymatic mechanisms (auto-oxidation of cholesterol molecules), with oxidation taking place in the sterol ring [6]. Generally, reactive oxygen species (ROS) are involved. Molecules such as singlet oxygen, hydrogen peroxide, hydroxyl radical, and ozone, oxidize the cholesterol molecule in lipoproteins and cell membranes, but also in food [10][32]. This reaction can promote the abstraction of an allylic hydrogen atom at C-7, and this carbon atom can easily react with molecular oxygen, forming a cholesterol peroxyl radical (COO-) [26].

Some oxysterols such as 7-ketocholesterol (7-KC), 7β-HC, 25-HC, and 7α-HC can be generated by both pathways [32]. A schematic representation of enzymatic and non-enzymatic pathways is shown in **Figure 2**.

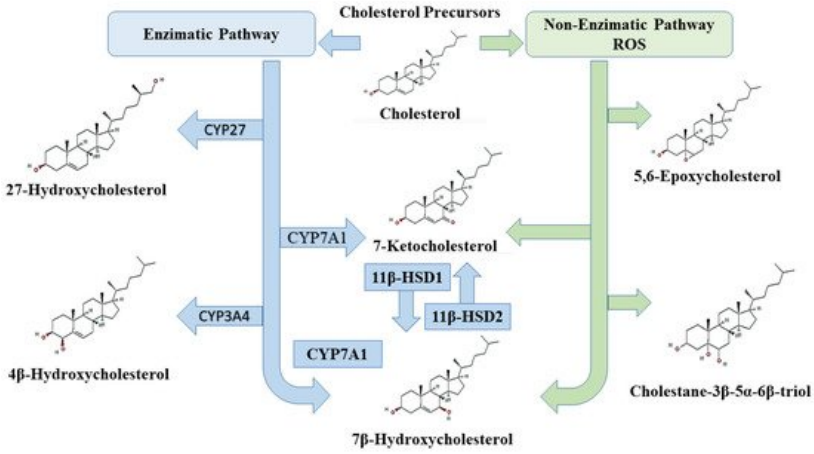

Figure 2. Schematic representation of synthesis of some oxysterols via enzymatic and non-enzymatic pathways.

Oxysterols can also be formed in food during heating or prolonged storage of cholesterol-containing products. High oxysterol levels can be found in several foods, such as powdered milk, cheese, egg products, and meat. Some oxysterols can also be formed from cholesterol in the stomach where all the conditions are in place to promote cholesterol oxidation: the acidic pH, and the presence of oxygen, iron ions, or metmyoglobin from dietary origin, all produce a highly pro-oxidizing environment [33]. Humans can absorb oxysterols from food into the bloodstream, where they are rapidly cleared from the plasma and re-distributed to different tissues of the body [34]. Tissues and organs may take up oxysterols from the plasma several times faster than cholesterol [35]. However, oxysterols are present in mammalian tissues at very low concentrations [19]. The main oxysterols commonly identified in human plasma include 7α-HC, 24(S)-HC, 4α-HC, and 4β-HC [36].

Oxysterols have been identified as a class of highly relevant signaling molecules that act in several human biological systems [1][22][35][37][38][39]. They play important roles in physiological and pathological processes, including cholesterol homeostasis, immune system regulation, platelet aggregation, inflammation, cell differentiation and proliferation, osteoporosis, age-related macular degeneration, atherosclerosis, cardiovascular disease, neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, and in the development and progression of some cancers [40][41][42].

Oxysterols can activate or inhibit several cellular signaling pathways [43] acting on cellular receptors, including nuclear receptors, a large superfamily of 48 ligand-inducible transcription factors. These proteins act as intracellular receptors that bind to lipophilic ligands capable of crossing the plasma membrane [44]. Of the nuclear receptors, the liver X receptors (LXRs) α and β are members of the nuclear receptor superfamily that regulate cholesterol homeostasis [43][45]. Oxysterols act as ligands of LXRα (NR1H3) and LXRβ (NR1H2) regulating the transcription of specific genes. LXRα is expressed primarily in the liver, intestine, adipose tissue, macrophages, and adrenal gland, whereas LXRβ is expressed in many cell types [17]. Upon binding to oxysterols, these transcription factors form obligate LXR–retinoid X receptor heterodimers, which interact with DNA to regulate the transcription of target genes [46]. Many LXR target genes are involved in cholesterol and fatty acid metabolism, including ABCA1, ABCG1, SREBP-1c, and fatty acid synthase. Other targets, such as AIM/SPa, are involved in the regulation of apoptosis and innate immune responses [45].

In addition, the role of oxysterols in cell death and differentiation processes is gaining attention. Many studies have shown that oxysterols exhibit cytotoxicity in several cells, including vascular cells (smooth muscle cells, vascular endothelial cells, and fibroblast) [10][41][47][48] and nerve cells (glial and microglial cells, and neurons) [41][48].

3. Oxysterols and Cell Death

Cell death is an important process for maintaining organism homeostasis. It eliminates old and injured cells, arising after cell damage or triggered by specific signaling. Cell death can be described broadly as an irreversible degeneration of vital cellular functions, ending with a loss of cellular integrity. This loss of integrity can be characterized by fragmentation or the permanent permeabilization of the plasma membrane [49]. This process is highly organized and crucial to normal physiological processes, such as embryonic development and tissue renewal. Cell death is also implicated in several other mechanisms, including the maintenance of epithelial barrier function, adaptative immune responses, recycling of biologic macromolecules, intracellular signaling, and preservation of genomic integrity [50]. However, it can also be involved in several pathological responses, such as cancer, cell injury and response to infectious pathogens.

A Nomenclature Committee on Cell Death (NCCD) was created to formulate guidelines for the definition and interpretation of cell death, taking into consideration its morphological, biochemical, and functional characteristics. With new analytical methodologies, novel mechanisms of cell death and new cell death pathways have been described [49].

The first reports on oxysterol-induced toxicity came out in the 1970s. Interest has grown since that time, with studies aiming to elucidate the mechanism that involves oxysterols and their cytotoxic action [51]. Because of the large number of ways that cholesterol can undergo oxidation, more than 60 different oxysterols have been reported. Despite this variety, only some of these oxysterols have cytotoxic properties. Some of them are, in fact, potent inducers of cell death [52]. Oxysterols can influence cancer progression and can act either as oncometabolites or as tumor suppressors based on the tumor microenvironment [53].

Generally, oxysterols are pro-apoptotic or pro-autophagic, but in high concentrations they can induce necrosis in some cell lineages [54]. However, the effects that oxysterols exert on cells are dependent on the cell lineage and the type of oxysterol, and on its concentration [2][19]. The main types of cell death promoted by oxysterols (apoptosis, autophagy, and necrosis) are described below and are schematically shown in **Figure 3** [55].

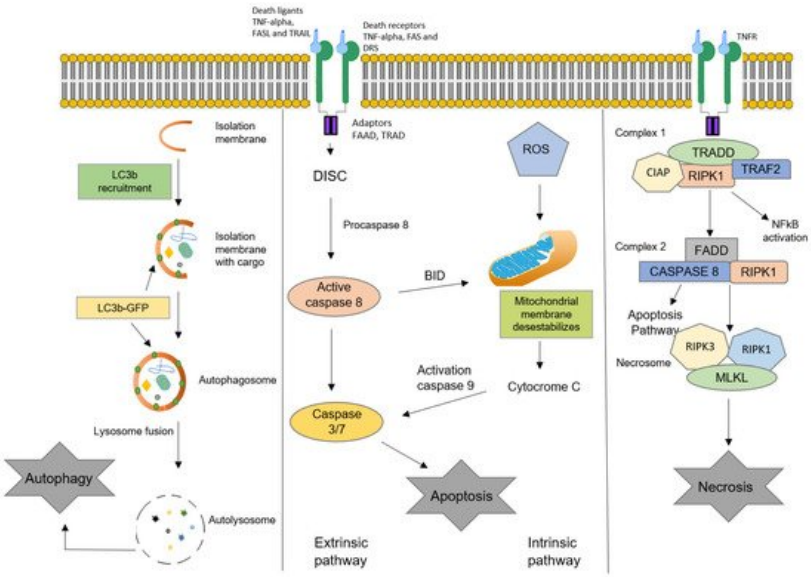

Figure 3. Schematic representation of the three main types of cell death: autophagy, apoptosis, and necrosis. TNF (tumor necrosis factor); FAS (CD95); FAS-L (CD95-ligand); TRAIL (TNF-related apoptosis-inducing ligand); TNFR (TNF receptor); ROS (reactive oxygen species); FADD (Fas-associated death domain); TRADD (TNFR-associated death domain); RIPK (receptor-interacting protein kinase); MLKL (mixed lineage kinase domain-like protein); DISC (death-inducing signaling complex).

3.1. Apoptosis

Apoptosis (from a Greek word meaning “falling off”, like leaves from a tree) is a form of programmed cell death [56][57][58] involving a mechanism of self-inflicted death encoded in the genetic material of cells [50]. Kerr et al. first described apoptosis, also known as programmed cell death, in the early 1970s [16][57][59][60]. Since its discovery, apoptosis has been one of the most studied biological processes of cellular death [61]. Unlike necrosis, a cell triggers apoptosis and follows a course toward death upon the detection of certain stimuli [61]. Apoptosis is associated with: (a) activation of mitochondrial permeability, with loss of transmembrane mitochondrial potential and release of cytochrome c into the cytosol; (b) activation of caspases; (c) condensation of chromatin (pyknosis); (d) endonuclease activation followed by internucleosomal DNA cleavage (multiple of 180–200 pairs of bases); (e) segregation of nucleoli; (f) nucleus fragmentation and/or condensation; and (g) blebbing of plasma membrane associated with the formation of apoptotic bodies [59][61].

Two apoptosis pathways have been described: the intrinsic and extrinsic. Intrinsic pathway signaling is linked to mitochondria function (mitochondrial pathway) [62][63]. ROS, in particular hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and superoxide anion (O_2^-), are toxic byproducts of oxidative phosphorylation. ROS is involved in the oxidative damage of mitochondrial lipids, DNA, and proteins, making mitochondria even more prone to ROS production. In turn, damaged mitochondria release high levels of Ca^+ and cytochrome c into the cytosol, triggering apoptosis [64].

The extrinsic pathway (death receptor pathway) is triggered by cell surface receptors that are stimulated by extracellular death-inducing signaling [62][63]. It has been proposed [62] that in the extrinsic pathway, extracellular signals or stress lead to the prompt release of ligands such as tumor necrosis factor (TNF), CD95-ligand (CD95-L or Fas-L), TNF-related apoptosis-inducing ligand (TRAIL or Apo2-L), and TNF-like ligand 1A (TL1A), which can bind to the death receptors. These death receptors are members of the TNF family and include TNF receptor-1 (TNFR-1), Fas, Apo-1, and TRAIL receptors (TRAIL-Rs). This receptor–ligand binding leads to the recruitment of the procaspase-8 enzyme to the death-inducing signaling complex (DISC). At the cytoplasmic end of the death receptor, adaptor proteins, such as the Fas-associated death domain (FADD) or TNFR-associated death domain (TRADD), are recruited. This recruitment results in the dimerization and activation of caspase-10 and caspase-8 monomers and, ultimately, in the dimerization of caspase-8 and the subsequent activation of the effector caspases-3/6/7 [62].

3.2. Autophagy

Autophagy is a normal cellular process involving intracellular degradation of cytoplasmic components, including organelles, proteins, and lipids. These components are sequestered inside vesicles (autophagosomes), formed by a double membrane, and delivered to the lysosome for degradation [65][66][67], providing substrates and energy [67].

The term autophagy was first used by Christian De Duve, the discoverer of lysosomes and peroxisomes and the first scientist to conduct experiments to demonstrate the biochemical involvement of the lysosomes in this process [68]. In 2016, Yoshinori Ohsumi received the Nobel Prize for Physiology and Medicine for his discovery of the mechanisms of autophagy using yeast as a model. One of his most important findings was the role of the ubiquitin-like proteins (UBLs) Atg5, Atg12, and Atg8 in the formation of the double-membrane vesicle (autophagosome), which is the functional unit of autophagy [66].

In summary, two UBL systems are involved in autophagosome formation. The first stage is the formation of a covalent link between Atg12 and Atg5 through to Atg7 (E1-like enzyme) and Atg10 (E2-like enzyme). The second stage is the formation of a covalent link between the microtubule-associated protein light chain-3 (LC3) and phosphatidylethanolamine (PE) through Atg7 and Atg3 (E2-like enzyme). The autophagosome then fuses with the lysosome, causing out the disintegration of the inner autophagosomal membrane and degradation of autophagosome content by lysosomal enzymes. This process provides amino acids, free fatty acids, and other products that cells can use for other purposes [69].

Autophagy is involved in several pathophysiological processes such as cancer, neurodegenerative disease, aging, autoimmune diseases (such as Crohn's disease and rheumatoid arthritis), heart disease, and infection, and can be a mechanism of caspase and apoptosis-independent cell death. In fact, the ability of autophagy to modulate cell death makes it a therapeutic target (through either up- or down-regulation) for several conditions such as cancer and neurodegenerative diseases [70].

However, autophagy can be triggered in any cell stimulated by stress or nutrient deprivation. Autophagy can be activated when cellular components are damaged, providing cells with molecular raw materials and energy [70][71][72]. Thus, autophagy can be considered to be a kind of internal quality control of organelles and proteins in cell machinery, with an important role as a survival mechanism [67]. Considering all the cellular processes involved, autophagy can exhibit pro-death or pro-survival functions. The pro-survival functions of autophagy are related to its support for cells in dealing with stress, clearing damaged proteins, organelles, pathogens, or aggregates, or providing the cell with energy and anabolic products during starvation [70].

3.3. Necrosis

Necrosis is an uncontrolled mechanism of cell death that occurs in response to extreme cellular injuries and trauma [50][73][74]. Necrosis can be induced by physical or environmental factors such as ischemia [50][73], infections, toxins, mechanical trauma, or thermal damage from extremely high or low temperatures [74]. Some tissues during inflammation or infection processes can secrete cytokines that can initiate necrosis pathways [75]. It could be considered as a failure of the cellular homeostatic process [50][73], leading the cell to premature death by autolysis [74]. The necrosis process involves disruption of the plasma membrane, followed by the release of cell contents into the extracellular space, triggering the recruitment of neutrophils, macrophages, and other elements of an acute inflammatory response [73][74]. Cells recruited in the necrosis process eliminate the dead cells and their products by phagocytosis [74].

Some studies have demonstrated that necrosis can apparently be a programmed and regulated form of cell death. Different types of necrosis have been identified, including programmed necrosis such as necroptosis, pyroptosis [56], ferroptosis (iron-dependent cell death) [56][76], mitotic catastrophe, and autophagic cell death [56].

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