Chapter 1

Introduction and Review of Literature on Cross-Talk between Autophagy and Apoptosis*

Abstract

The definition for autophagy holds a 'single' meaning as a conserved cellular process that constitutes a recycling pathway for damaged organelles and long-lived proteins to maintain nutrient homeostasis and mediate quality control within the cell. But this process of autophagy may behave ambiguously depending on the physiological stress as the stress progresses in the cellular microenvironment; the 'single' meaning of autophagy changes from the 'cytoplasmic turnover process' to 'tumor suppressive' and, to a further extent, 'tumor promoter' process. In a tumorigenic state, the chemotherapy-mediated resistance and intolerance due to upregulated autophagy in cancer cells have become a significant concern. This concern has provided insight to the scientific community to enter into the arena of cross-talk between autophagy and apoptosis. Recent findings and ongoing research have provided insights on some of the key regulators of this cross-talk; one of them is Beclin1 and their involvement in the physiological and pathophysiological processes. However, reconciliation of these two forms of death remains an arena to be explored extensively. This chapter sheds light on the interplay between autophagy and apoptosis, emphasizing one of the key players, Beclin1, and its importance in health and diseases. The chapter also defines the gap in the literature and the scope of the current work.

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1.1 Introduction to Autophagy and Apoptosis

Life depends on a delicate balance between anabolism (synthesis) and catabolism (degradation). Two of the most fundamental processes in the cell, autophagy, and apoptosis, constitute the self-destruction processes that lead to the clearance of superfluous, damaged, and no longer necessary cells and organelles. Upon going away from the usual track of these processes, the outcome can be terrible and become the principal cause of demise and disability in many diseases, including cancer, neurodegenerative, cardiovascular, and Infectious diseases. Even knowing about the unquestionable significance of the complex and well-controlled process of cellular degradation through autophagy and apoptosis, our understanding of coactions between these two processes and their regulation remains to be uncovered for the recent research supporting the cross-talk between these two inter-regulatory processes during stress responses and other pathophysiological conditions (Kim and Lee 2014).

Apoptosis (also known as type I cell death) is a well-studied example of programmed cell death, which is conserved from *Caenorhabditis elegans* to humans. It plays an essential role in vertebrates during development, cellular homeostasis, and tumor suppression (Ke *et al.* 2018), (Luke *et al.* 2003). This type of cell death classically implies itself due to its morphological description, which involves a signaling cascade wherein the activation of executioner caspases leads to the clearance of intracellular components in an accelerated manner and culminates in the phagocytosis of the dying cell (Chipuk and Green 2005). In more detail, based on whether the initiation signal received for the cell's death is an external stimulus or internal stimulus, apoptosis is mediated via two pathways- the intrinsic pathway (mitochondria-mediated pathway) and the extrinsic pathway (Figure 1.1).

Another self-degradation process that has attracted attention in the research arena is autophagy. The general definition of autophagy follows the degradation pathway that allows

the delivery of degrading cellular components to the lysosome. The most prevalent form of these processes is macroautophagy, hereafter referred to as autophagy, which has been found to be highly conserved in all eukaryotic cells ranging from yeast to mammals. In eukaryotes, autophagy plays an essential role during development and maintaining cellular homeostasis through the degradation of proteins and intracellular organelles. Autophagy is a highly regulatory process and is associated with multiple conserved characteristics that constitute sequestration of the cytosol and intracellular organelles by a cytosolic double-membrane vesicle termed as an autophagosome which culminates in the fusion of the inner vesicle or autophagic body with the lysosome and results into the hydrolytic degradation of cargo and recycling of the constituents (Figure 1.1).

Autophagy takes place even in normal cells, where this process is responsible for the turnover of cell constituents. Still, the process is induced in cells subjected to physiological remodeling or ill-treated by some pathological means. This induced autophagy holds the extreme form of autophagy, referred to as autophagic cell death (also known as type II cell death), which leads to self-cannibalization of the cell and ultimately to cellular demise. Scientists have identified this non-apoptotic form of programmed cell death as a cause of physiological remodeling during development and differentiation and demolishment in many diseases, including cancer, neurodegenerative, cardiovascular, diabetes, liver diseases, and micro viral infections. The relationship between both forms of death is complex and circumstantial. Generally, it seems complicated to delineate whether both processes, i.e., an apoptotic form of death and autophagic cell death, act together or mutually exclusively to give their effects on the cell. Intensive research indicates that perhaps based on the nature, amplitude, and duration of the stimulus and threshold of the stressor, the cell adapts itself to either form of cell death (Figure 1.2).

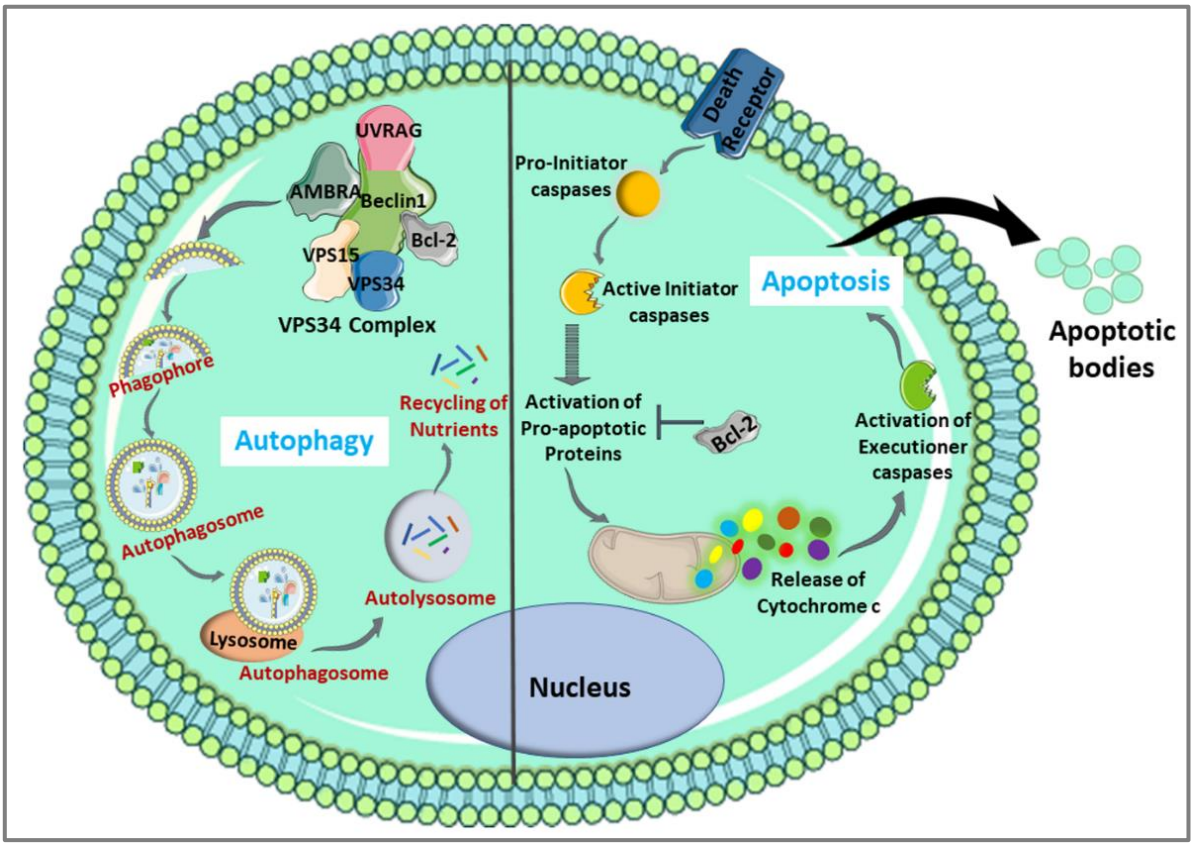

Figure 1.1. The process of Autophagy and Apoptosis. The Autophagy. This process starts with the activation of the VPS34 complex. The key protein of this complex and key regulator of the process of autophagy is Beclin1. Upon activation of this complex, the formation of an isolation membrane (referred to as phagophore) starts, which encloses the organelles and the proteins to be degraded and forms a structure called an autophagosome. This structure fuses with the lysosome to form a structure termed an autolysosome. The lysosomal enzymes degrade the components present within this structure, and ultimately, the nutrients and metabolites are recycled. Another form of cell death is apoptosis. The extrinsic apoptosis pathway. The programmed cell death can be initiated via two routes, the extrinsic and intrinsic pathways of apoptosis. The extrinsic pathway of cell death is triggered with the ligation of the death receptor, which leads to the activation of several initiator caspases. These initiator caspases, upon activation, activate several proteins in the path and ultimately join with the intrinsic apoptotic pathway and lead to the release of cytochrome c from the mitochondria. This release culminates in the activation of executioner caspases and, finally, apoptotic cell death.

The tight regulation of autophagy depends upon a group of evolutionarily conserved genes, the *Atg* (autophagy-related) genes (Wang and Klionsky 2003). The key regulatory protein of this autophagic pathway, Bcl-2-interacting protein1 (Beclin1), interacts with the several binding partners of the autophagy process as well as regulatory proteins of the apoptotic cell death pathway and plays an essential role in the regulation of the cross-talk between these two forms of cell death and leads to regulation of wide array of physiological and pathophysiological conditions (Liang *et al.* 1998).

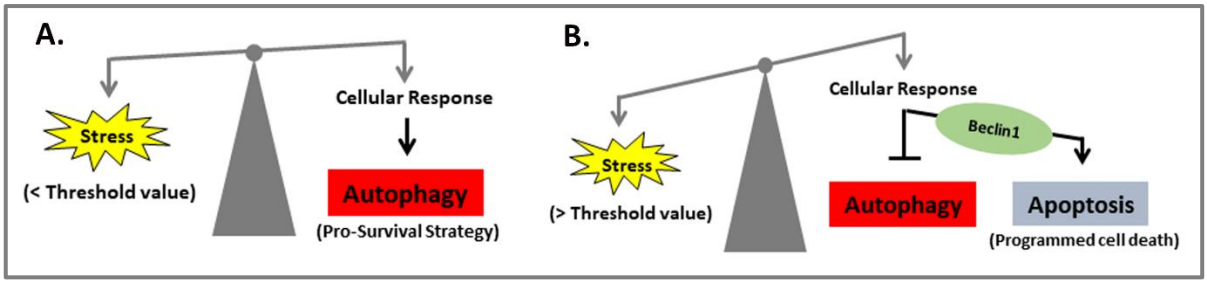

Figure 1.2. Stress-response balance. The two forms of cell death as a cellular response is dependent on the threshold of stress within the cellular microenvironment. **(A)** The cell executes autophagic cell death when it faces below threshold level stress conditions. This adaptive autophagy has cytoprotective behavior and acts as a barrier against stress, and prevents the process of apoptosis from being initiated within the stressed cell. **(B)** As stress progresses beyond the threshold value, the cell's behavior becomes tumorigenic, and the tumor cell starts using autophagy as a tumor-survival mechanism to meet its exaggerated cellular requirements. At the same time, some of the apoptotic proteins play a crucial role in switching the process of cell death from autophagy to apoptosis in the highly stressed microenvironment and, hence, in tumor clearance.

Extensive research in this field has raised the possibility of using genetic and therapeutic approaches to inhibit autophagy, which culminates in the transition of pathologically ill-treated cells from the pro-survival autophagic state to apoptotic death. These approaches have been well-studied in different forms of cancer. Although these findings are not very well established in the clinical utilities, they hold an exciting and promising approach in this emerging field of a synergistic form of cell death.

In this chapter, we discuss the regulatory mechanism behind the interplay between these circumstantial and complex forms of cell death, autophagy, and apoptosis by

emphasizing some key regulatory proteins involved in these respective pathways and also, we elaborate the causative role of the synergistic action of these cell death forms in mammalian physiology and its associated diseases.

1.2 Autophagy promotes cellular survival in normal growth conditions

The autophagic or lysosomal degradation pathway is one of the two major regulated protein pathways and is the only known pathway for organelle degradation in eukaryotic cells. The primary function of this process is to degrade intracellular components to recycle those degraded components; it acts as the building block for the synthesis of new macromolecules (Figure 1.3). The maintenance of cytoplasmic turnover is not only limited to cells but is also maintained in the varieties of tissues and organs or the whole body through the endocrine mechanism of cell signaling (Kim and Lee 2014, Wang et al. 2019b).

1.2.1 Roles of Autophagy in Whole Body Metabolism

Under normal physiological conditions, autophagy occurs at a basal level to maintain cellular homeostasis. The hypothalamus, a small area in the brain, is responsible for stimulating essential regulatory metabolic functions of the body and has gained the attention of scientists for investigating the role of autophagy in cellular homeostasis. Mediobasal hypothalamus-specific Atg7 gene knockout mice show weight gain due to hyperphagia and decreased energy expenditure, followed by obesity and insulin resistance after giving a high-fat diet. These observations suggest that autophagy in the hypothalamus plays an essential role in energy homeostasis (Meng and Cai 2011). Induced autophagy is also evident in post-injury microtubule dynamics and axon regeneration; thereby, it is suggested as a cytoprotective mechanism in remodeling the cytoskeleton structures in the injured neurons (He *et al.* 2016).

Hepatocyte autophagy has been widely studied, as the liver plays a significant role in metabolism and its associated pathophysiology. The hepatocyte-specific Atg7 gene-knockout

(Atg7Δhep) mice have shown increased lipid content in the liver in both the fed and fasted states (Singh *et al.* 2009). In another experimental condition, hepatocyte-specific Vps34-knock-out (Vps34Δhep) mice show the build-up of the lipid content in the liver (Jaber *et al.* 2012). Furthermore, hepatic autophagy has also been studied in the context of insulin sensitivity and type 2 diabetes mellitus. Mice with obesity-induced insulin resistance show obstructed autophagy that could either be attributed to reducing the expression of some of the key ATGs or the calpain-mediated cleavage of the core autophagy complex.

In vivo studies have demonstrated the role of autophagy in insulin-producing pancreatic β cells. The β cell-specific Atg7 gene has been knocked out (Atg7Δβ cell) in mice. These mutant mice have shown a reduction in β cell mass and insulin secretion, which led to hyperglycemia, glucose intolerance, and hypoinsulinemia (Ebato *et al.* 2008). The cytoprotective role of Beclin1-mediated autophagy is also highlighted in adipose tissue homeostasis, the failing of which results in the development of severe lipodystrophy (Jin *et al.* 2021). Similarly, Beclin1-dependent autophagy is also found as a cytoprotective approach in improvising the biochemical and clinical phenotypes of urea cycle disorders such as ornithine transcarbamylase (OTC) and argininosuccinate lyase (ASL) deficiencies (Soria *et al.* 2021).

Furthermore, the constitutive autophagy process has also been studied in skeletal muscle. As skeletal muscle actively participates in insulin-mediated glucose utilization and adversely in the pathogenesis of type 2 diabetes mellitus, scientists have studied the effect of autophagy in skeletal muscle-specific Atg7 gene knocked out (Atg7Δsm) mice for glucose utilization or insulin sensitivity (Kim *et al.* 2013). These mice are found to have reduced lean body mass and fat mass accompanied by increased glucose clearance and energy expenditure. Moreover, the mutant mice are found to have atrophied muscle and shown decreased muscle force, suggesting the role of autophagy in preserving the muscle mass (Masiero *et al.* 2009).

Likewise, the role of exercise-induced autophagy in the skeletal muscle has also been studied in mice with Bcl2 knock-in mutations of three different alanine residues (Bcl2 AAA), which led to the inhibition of dissociation of Bcl2 from Beclin1 and that of activation of autophagy. The basal autophagy is found intact in these mutant mice, but an individual's exercise-induced autophagy and capacity to exercise are impeded. Following this, exercise-trained mice haploinsufficient for Beclin1 (Becn1+/-) have shown an attenuated increase in basal autophagy and lessened improvement in endurance capacity, suggesting the role of basal autophagy in skeletal muscle adaptation during endurance exercise training (He *et al.* 2012).

In the context of the lymphoid system, several investigations have been carried out to decipher the role of autophagy in antigen presentation and T-cell development. MHC class II molecules are among the two major classes of MHC molecules and participate in the endogenous pathway of antigen presentation majorly in B cells and fibroblasts. An epitope derived from the cytosolic protein neomycin phosphotransferase II (NeoR) gets sequestered into the autophagosomes and subsequently fuses with the endosome/lysosome to be processed into the antigenic peptides and presented on the cell surface. This process defines a convergent action of autophagy and MHC class II-restricted antigen presentation. Furthermore, epithelial cells of the thymus are supposed to present self-antigens to lymphocytes to select positive- and negative-T cells. As there is no phagocytic activity in the thymic epithelial cells, it may be said that the intracellular cytoplasmic components are degraded and presented as self-antigens on the cell surface through the autophagic process. This evidence may suggest the role of thymic epithelial cell autophagy in T-cell development and central tolerance. The recent finding also indicates the role of Beclin1 *in vivo* and *in vitro* melanogenesis (Rai *et al.* 2020). Altogether, these numerous roles of Beclin1 suggest its independent attributes other than autophagy and apoptosis.

Table 1.1. Effect of key regulators-mediated autophagy on the physiology of the body.

Location/Process	Level of Autophagy	Effect of Autophagy	Gene Involved	Model Organism	Overall Effect after Genetic/Pharmacological Inhibition of Autophagy	Ref.(s)
Whole body metabolism						
Hepatocyte	Basal level	Lipid metabolism	*Atg7, Vps34*	Mice	Increased lipid content in the liver; Obesity; Insulin resistance	(Singh *et al.* 2009, Jaber *et al.* 2012)
Hypothalamus	Basal level	Cellular homeostasis	*Atg7*	Mice	Weight gain; Decreased energy expenditure; Obesity; Insulin resistance	(Meng and Cai 2011)
Pancreatic β cells	Basal level	Cytoprotective role	*Atg7*	Mice	Hyperglycemia; Glucose intolerance; Hypoinsulinemia	(Ebato *et al.* 2008)
Development and differentiation processes						
Fertilization	Induced	Paternal mitochondria degradation in sperm	-	-	-	(Rojansky *et al.* 2016)
Embryogenesis	Induced	Cytoplasmic turnover; Autophagy-dependent cleavage	*becn1, Vps34*	Mice	Defective embryogenesis; Ceased developmental growth	(Yue *et al.* 2003)
Blastocyst stage	Induced	Blastocoel formation	*Atg5, becn1*	-	Failure in embryonic cavitation; Formation of embryoid bodies with persisted cell corpses	(Qu *et al.* 2007)
Embryonic stem cells (ESCs)	Upregulated; Increased ciliation-dependent-induced autophagy	Differentiation of blastocyst's inner cell mass to form embryonic stem cell; Pleuripotency of embryonic stem cell; Directs hESCs toward	*becn1*	-	-	(Ou *et al.* 2014, Gregory *et al.* 2016, Jang *et al.* 2016)

		neuroectoderm fate instead of mesendoderm fate				
induced Pluripotent stem cell (iPSCs)	Balanced Autophagy	Pluripotency of iPSCs; Somatic cell reprogramming	-	-	Bafilomycin (an autophagy inhibitor)-dependent induction in cell death; High concentration of Rapamycin (an autophagy activator)-dependent differentiate into EBs; Appropriate concentration of Rapamycin-dependent elevation in the reprogramming efficiency of somatic cells	(Sotthibundhu *et al.* 2016)
Initial trimester of pregnancy	Induced autophagy	Invasion of extravillous trophoblast (EVT) cells of the embryo; Vascular remodeling of EVT; Pro-survival function	-	-	Soluble endoglin-mediated autophagic inhibition leads to suppressed EVT cells invasion	(Nakashima *et al.* 2013)
Organogenesis	Induced autophagy	Cerebral corticogenesis	*Vps34*	Mice	Positional defects in excitatory neurons; Delayed axon extension	(Inaguma *et al.* 2016)
Human mammary epithelial cells	TRAIL-dependent induction in autophagy	Post-lactational involution	-	-	-	(Teplova *et al.* 2013)

1.2.2 Roles of Autophagy in the Development and Differentiation Processes

Several growing pieces of evidence suggest the role of autophagy in the mammalian life cycle during its various distinct developmental stages. During fertilization, the mitochondria in the sperm need to be degraded. Several studies demonstrated that this paternal mitochondrial degradation could be due to autophagy (more precisely, mitophagy) (Rojansky *et al.* 2016), but the investigations remain elusive. After fertilization, autophagy also shows its undeniable significance when it comes to cleavage, which is supported by many experimental studies. Recent findings indicate the role of ATGs during the cleavage processes, which demonstrate the mouse embryos with becn1-/- and vps34-/- show defective embryogenesis (Yue *et al.* 2003). This study suggests that due to the lack of cytoplasmic turnover provided by autophagy, developmental growth ceased.

Once the cleavage process attains the blastocyst stage, several roles of autophagy have been suggested at this level. Studies reveal that blastocoel formation (a cavity in the trophoblast) is partially due to autophagic cell death (induced autophagy). Cells lacking the autophagy genes, Atg5 or beclin1, fail in embryonic cavitation; instead, they form embryoid bodies (EBs) with persistent cell corpses. Such EBs are with a downregulated "eat-me" signal (phosphatidylserine) and a "come-get-me" signal (lysophosphatidylcholine). This observation suggests an interplay between autophagy and apoptosis during the developmental processes (Qu *et al.* 2007).

The blastocyst's inner cell mass (ICM) is *in vitro* cultured and referred to as embryonic stem cells (ESCs). Upon differentiation and under oxidative stress, the basal autophagy in these ESCs is observed to be upregulated through the class-III PI3K/beclin1 (BECN1) and mTOR pathways (Ou *et al.* 2014). Moreover, the primary cilium, present on the surface of the ESCs, is responsible for maintaining the pluripotency of the ESCs through coordination with autophagy (Gregory *et al.* 2016). One study has also demonstrated that during the

differentiation of human embryonic stem cells (hESCs) to neuroectoderm or mesendoderm fate, an increase in the ciliation induces autophagy, which directs hESCs toward neuroectoderm fate instead of mesendoderm fate (Jang *et al.* 2016). Consistent with these findings, a study has reported that to maintain pluripotency, mouse ESCs have been observed to exhibit high autophagic flux (upregulated autophagy) compared to somatic cells (Liu *et al.* 2017).

Moreover, the role of autophagy has been studied in induced pluripotent stem cells (iPSCs), which suggests that the pluripotency of iPSCs depends upon the intensity of autophagy. In a study, treatment of iPSCs with bafilomycin (an autophagy inhibitor) induces cell death, while that of with a high concentration of Rapamycin (an autophagy activator) leads to differentiation into EBs (Sotthibundhu *et al.* 2016), and intriguingly, treatment with appropriate concentration of Rapamycin elevates the reprogramming efficiency of somatic cells. This suggests that either too low or too high an intensity of autophagy deteriorates the pluripotency of iPSCs; instead, balanced autophagic flux is required for somatic cell reprogramming.

Exceeding the role of autophagy in the developmental processes has essential importance in embryo implantation and during embryonic development. Several *in vitro* and *in vivo* experimental findings have shown that during the initial trimester of pregnancy, the low-oxygen and low-nutrient environment of the uterus induces the autophagic pathway in the extravillous trophoblast (EVT) cells of the embryo. It has also been reported that soluble endoglin-mediated autophagic inhibition leads to suppressed EVT cell invasion (Nakashima *et al.* 2013). Another study demonstrates that upon exposure to physiological hypoxia condition, the placental trophoblasts show upregulated autophagy as a pro-survival function to oppose the hypoxia-induced apoptotic cell death in the EVTs of early placental tissues (Chen *et al.* 2012). These studies together suggest the essential participation of autophagy in

the physiological hypoxia-induced function, invasion, and vascular remodeling of EVT, which are necessary for the first stage of placentation.

After successful implantation, embryo development starts coming into the picture, and likewise, the essential role of autophagy has been observed to promote organogenesis during embryonic development. In mouse cerebral corticogenesis, loss of Vps34 protein shows positional defects in excitatory neurons and delayed axon extension (Inaguma *et al.* 2016). In embryonic cardiac development, autophagy shows a pivotal role; as in a study, it has been reported that Atg13-deficient embryos exhibit impaired autophagosome formation and enhanced sensitivity to tumor necrosis factor α (TNF-α)-induced apoptosis (Kaizuka and Mizushima 2016). The role of autophagy has also been studied in the embryonic development of bone tissues. According to the study reports, induced autophagy has been observed in terminal chondrocyte differentiation upon exposure to a low-oxygen environment in the absence of a blood supply. Likewise, the active participation of autophagy has also been deciphered in mouse embryonic fibroblasts (MEFs) and primary lung fibroblasts. It has been reported that an immunoregulatory factor, Interleukin (IL)-2, favors the interaction of high mobility group box1 (HMGB1) and Beclin1 and induces autophagy; thereby, it leads to cell growth in fibroblasts. However, the knockdown of *beclin1* results in obstructed IL-2-induced autophagy and enhanced apoptosis (Kang *et al.* 2013). These results suggest the pro-survival role of autophagy in the growth of fibroblast cells.

During all the trimesters of pregnancy, autophagy has been observed to play essential roles; a few of them are to help in the survival of oxygen-glucose-deprived cytotrophoblast cells, involve in amniotic membrane rupture during labor, and assist in the successful fetus delivery after completing full-term pregnancy; as a study has reported that beclin1-deficient ovarian granulosa cells lead to impaired progesterone production which culminates to preterm labor phenotype (Gawriluk *et al.* 2014). Increasing evidence regarding the function

of autophagy suggests that it carries out distinct functions even after the delivery of the fetus. Once the fetus is delivered, the sudden interruption of trans-placental nutrient supply leads to immediate upregulation in autophagy for 3-12 hours to adapt to this severe starvation. This elevated level of autophagy comes back to the basal level within 1-2 days of birth (Kuma *et al.* 2004).

Regardless of the nutritional status, autophagy plays a vital role in homeostasis and renovation in various tissues and organs in adults. Basal autophagy acts as an intracellular quality-control mechanism as it constitutes catabolic degradation of long-lived proteins and damaged organelles to maintain normal cellular function. Under a nutrition-rich environment, 1-1.5% of the cellular proteins get catabolized per hour by autophagy in the liver (Mizushima and Komatsu 2011). Moreover, the role of autophagy has also been observed in the transition from pro- to pre-B cells (Miller *et al.* 2008). Interestingly, autophagy also plays a pivotal role in defense mechanisms and removes invading intracellular pathogens like bacteria, viruses, and parasites. Several studies have been reported based on the potential roles of autophagy in the self-renewal and differentiation of adult stem cells. Autophagy has been found to play a pivotal role in the pluripotency of hematopoietic stem cells (HSCs) and in protecting them from metabolic stress (Warr *et al.* 2013), as a study has reported that loss of autophagy in HSCs leads to impaired self-renewal activity and regenerative potential (Ho *et al.* 2017). Together with these findings, autophagy has also shown its regulatory effect in the self-renewal, proliferation, and differentiation of hepatic progenitor cells (HPCs). A study demonstrates that knockdown of beclin1 in mouse HPCs results in compromised regenerative potential, whereas overexpression of Beclin1 restores the functionality of stem cells (Cheng *et al.* 2015). Likewise, human mesenchymal stem cells (hMSCs) also exhibit induced autophagy. In a study, it has been reported that autophagic inhibitor Bafilomycin A1 resulted in impaired autophagy in these cells. Consistent with this result, it has also been demonstrated

that the knockdown of anti-apoptotic protein Bcl-xL results in inhibited autophagy, which influences the survival and differentiation of adult hMSCs (Oliver *et al.* 2012), suggesting the inter-dependent role of autophagic and apoptotic proteins in maintaining the stemness of adult hMSCs.

Furthermore, several experiments have been performed to know about the role of autophagy in gametogenesis. Folliculogenesis describes the maturation process of the ovarian follicle, and autophagy has been observed to be involved in this process of oocyte development. A recent study has shown the Beclin1-mediated upregulated autophagy in the spermatozoa of infertile males, thereby suggesting the role of Beclin1 in spermatogenesis and the fertilizing ability of spermatozoa (Elzeiny *et al.* 2019).

Several experimental studies provide evidence of autophagic cell death during the developmental stages. On a few occasions, to make massive cell elimination, both the effects of autophagic and apoptotic forms of cell death have been observed. Human mammary epithelial cells undergo tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced autophagy along with caspase-mediated apoptosis for the *in vitro* formation of a hollow lumen (Mills *et al.* 2004). Moreover, it is not only limited to the developmental stage; instead, both forms of cell death have been reported in adulthood during mammary gland post-lactational involution (Teplova *et al.* 2013).

It is worth mentioning the undeniable importance of autophagy during the distinct stages of the mammalian life cycle (summarized in table 1.1). It plays a pivotal role in the pre-embryonic stage, followed by embryonic development, post-natal development, and gametogenesis. Further studies can be attempted to decipher the role of autophagy in mammalian physiology.

1.3 Autophagy in cancer

Adaptive autophagy or "autophagic cell death" is primarily marked by the formation of numerous autophagic vacuoles and is associated with the upregulated form of autophagic cell death (Chavez-Dominguez *et al.* 2020) (Figure 1.3). This upregulated form of cell death occurs during embryonic development and differentiation, exposure to physiological stress, or invasion of pathological means. This form of cell death is known as type II programmed cell death, which is non-apoptotic. It shows early degradation of organelles and the preservation of cytoplasmic elements until late stages. In contrast to apoptotic cell death, autophagic cell death shows caspase activation and DNA fragmentation at very late stages of cell death. The ambiguous role of autophagy in tumor biology remains controversial. Considering the several studies supporting the dual behavior of autophagy, we have tried to delineate the boundary between the facets of autophagy, viz. tumor suppressor, tumor promoter, and the transitional facet from autophagy to apoptosis in different tumor models (Table 1.2).

1.3.1 Autophagy in Stress (Below Threshold Value) Behaves as Tumor Suppressor

Below the threshold value of the stress, the upregulated autophagy tends to approach autophagic cell death in the chemotherapeutic agents-treated tumor cells. Several studies reveal the role of autophagic cell death in mammals treated with drugs, toxins, or stress stimulators. According to a study, it has been reported that MEFs that lack both the Bax and Bak proteins (pro-apoptotic members of the Bcl-2 family proteins) (Bax/Bak-/- MEFs), when treated with the apoptosis-inducing reagents such as; etoposide or staurosporine, shows a significant decline in the cell viability due to a non-apoptotic form of cell death, more precisely, the autophagic cell death. This autophagic cell death shows the characteristic presence of autophagosomes in the Bax/Bak-/- MEFs with considerable accumulation of one

of the key autophagy proteins Beclin1 and, notably, sustained activation of c-Jun N-terminal protein kinase 1 (JNK1) (Shimizu *et al.* 2010).

In a recent study, the involvement of Beclin1 has been validated to behave as a tumor suppressor. In this study, upon lipopolysaccharide-induced septic shock, myeloid-specific inhibition of Beclin1 has been observed correlating with spontaneously developed precursor B cell lymphoma in mice; hence, suggesting the tumor-suppressive facet of myeloid Beclin1 (Tan *et al.* 2019). Considering the similar role of Beclin1, another study on human meningiomas shows a correlation of higher expression of Beclin1 with better prognosis, lower pathological grade, and more prolonged survival of the patients with meningiomas, indicating the tumor-suppressive role of Beclin1 in intracranial tumors (Kuo *et al.* 2019). Another *in vivo* study reveals a correlation of Beclin1 overexpression with a decline in cell proliferation in human esophageal carcinoma, resulting in a lower tumor growth rate in mice, suggesting the tumor suppressor role of Beclin1-mediated induced autophagy in esophageal carcinoma (Zhang and Dong 2019).

Furthermore, resveratrol, a natural phytoalexin with antineoplastic properties, is observed to inhibit growth with indicative characteristics of apoptotic cell death and induce autophagic cell death in human ovarian carcinoma cell lines. This study reveals the mechanical action of resveratrol to cause non-apoptotic cell death in ovarian carcinomas and proposes itself as an effective pharmacological drug to treat human ovarian cancer that shows chemoresistance due to ineffective apoptosis (Opipari *et al.* 2004).

Consistent with this finding, fenretinide, an example of a synthetic derivative of retinoic acid, shows an elevated expression of Beclin1, which results in the induction of autophagic cell death in the MCF-7/7.0.3 cell line. Hence, fenretinide can be utilized as a promising drug to overcome chemoresistance due to defective apoptosis to improve cancer therapy (Fazi *et al.* 2008).

Autophagic cell death has been proposed to play a role in eliminating tumor cells via the caspase-independent cell death pathway. In this regard, one of the mechanisms behind this caspase-independent cell death has been suggested by scientists. They have found that dysregulated H-Ras activity-mediated oncogenic microenvironment shows a marked upregulation in the expression of Beclin1 and Noxa (a BH3-only protein)-mediated dissociation of Beclin1 and a Bcl-2 family member protein (Mcl-1). These events together indicate the induced autophagic cell death to circumvent the oncogenic environment imposed due to dysregulated Ras signals in the cancer cells (Elgendy *et al.* 2011).

Furthermore, the human mammary carcinoma cell line (MCF-7/7.0.3 cells) lacking functional caspase-3 activity, upon treatment with the anti-estrogen drug tamoxifen, results in induced autophagic cell death with the characteristic presence of intact cytoskeletal elements, which is essential for the formation of autophagosomes. To find the underlying molecular mechanism behind this tumor suppressor facet of Beclin1-mediated autophagy, recently, Zhong et al. revealed deoxycytidine kinase-mediated downregulation in apoptosis and upregulation in autophagy by inhibiting the interaction of Beclin1 and Bcl-2 in ionizing radiation-treated breast cancer cell lines (Zhong *et al.* 2018). Similarly, inhibition of the interaction between Beclin1 and human epidermal growth factor receptor 2 (HER2) abrogates the tumorigenic effect of HER2 and leads to upregulation in the autophagy and, hence, renders a novel approach in the treatment of HER2-positive breast cancer (Vega-Rubín-de-Celis *et al.* 2018). In order to unravel the molecular mechanism behind the tumor suppressor facet of Beclin1 in breast cancer, Wijshake et al. revealed the correlation of increased cell surface localization of E-cadherin with Beclin1-mediated tumor suppression in MCF7 breast cancer cells (Wijshake *et al.* 2021). This study efficiently indicates a novel mechanism behind Beclin1-mediated restriction in tumor growth in breast cancer cells.

Consistent with this finding, similar observations have been found in other cellular settings, such as mouse L929 fibroblast cells and human U937 monocytoid cells, which show induced autophagic cell death with the presence of autophagic vacuoles upon treating the cells with zVAD (a caspase-inhibitor). Moreover, upon reduction in the Beclin1 protein, these cells show a decline in the formation of autophagic vacuoles and reduced zVAD-triggered autophagic cell death (Yu *et al.* 2004). These results together suggest that upon treating the cells with a bearable threshold of stress, the cells tend to induce autophagic cell death as an onco-suppressive action in order to circumvent the applied stress onto the cells.

Moreover, there are certain BH3 mimetics-dependent chemotherapies that have been targeted to inhibit the interaction of Beclin1 and Bcl-2. These BH3 mimetics-mediated chemotherapies bind to Bcl-2 and inhibit the interaction between Beclin1 and Bcl-2 complex. Several studies report that BH3 mimetics such as obatoclax, (-)-gossypol, ABT-236, and ABT-737 disrupt the Beclin1 and Bcl-2 interaction (formed on endoplasmic reticulum), and this disruption led to induction in the autophagic cell death. These studies suggest that BH3 mimetics-mediated induced autophagy acts as a tumor-suppressive behavior in apoptosis-resistant cells (Pedro *et al.* 2015). Together, these findings support the tumor suppressor role of Beclin1 in autophagy.

So far, we have been highlighting the autophagic cell death-dependent tumor suppressor role of Beclin1; however, scientists have also claimed the non-autophagic cell death-dependent tumor suppressor role of Beclin1, which includes endocytic receptor trafficking. In this study, Beclin1 accelerates the recruitment of hepatocyte growth factor tyrosine kinase substrate (HRS) to endosomes; thereby, it promotes the sorting of surface receptors to intraluminal vesicles to silence the signals and degrade the lysosomes. In the tumorigenic microenvironment, the low expression of Beclin1 leads to abrogation of this Beclin1-mediated endocytic receptor trafficking, thereby resulting in sustained receptor functions and

culminating in tumor progression. Hence, the involvement of Beclin1 in endocytic receptor trafficking is considered a non-autophagic cell death-mediated tumor suppressor facet of Beclin1 (Matthew-Onabanjo *et al.* 2020).

Considering all the findings that indicate the tumor suppressor role of Beclin1-mediated autophagy, one thing can probably be deduced that: when the normal cell senses the oncogenic microenvironment, the autophagy gets upregulated to prevent the cell from being converted to the tumor cell, and this suppression depends on the stress level imposed onto the cell.

1.3.2 Autophagy in Prolonged Stress Behaves as Tumor Promoter

There is generally reduced autophagic cell death in cancer cells than in their normal counterparts. Still, it is highly upregulated once these cancer cells are treated with radiation, chemotherapeutic agents, or exposure to other prolonged stress. Cancer cells utilize extensive autophagic degradation to generate metabolic and biosynthetic catabolites to fulfill the highly demanding nutritional requirement. These cancer cells acquire extensive autophagy to adapt to stressful environments, such as chronic inflammation, DNA damage, and genome instability induced by chemotherapy. Several findings support the stress-dependent tumor promoter behavior of autophagy.

An antitumor and alkylating agent, temozolomide (TMZ), at a clinically achievable dose (100 μM, three days), shows induced autophagy with the characteristic presence of autophagosome secondary lysosomes and development of AVOs in malignant glioma cell lines. However, these malignant glioma cells exhibit no significant apoptotic cells with or without treating these cells with TMZ (100 μM, three days) (Kanzawa *et al.* 2004). Furthermore, another antitumor pharmaceutical agent, arsenic trioxide (As2O3), has also been found to induce autophagic cell death in malignant glioma cells via upregulating a stress-associated protein, Bcl-2/adenovirus E1B 19-kDa-interacting protein 3 (BNIP3), with

the characteristic presence of autophagic vacuoles and AVOs which upon treatment with an autophagy-inhibitor (3-MA) result in suppressed development of AVOs, and dysfunctional mitochondria which is devoid of activated caspases (Kanzawa *et al.* 2005). In support of these findings, a recent study also suggests the role of Beclin1-mediated upregulated autophagy is dominant in the progression of high-grade gliomas (CJ *et al.* 2017). In line with this, the tumor-promoting activity of Beclin1-mediated autophagy is also correlated with the vasculogenic mimicry formation and metastatic ability of the cobalt chloride-induced hypoxic human glioma U87MG cells (Duan 2018). Together, these studies suggest that the antitumor agent-induced autophagy is utilized as a cell survival mechanism for malignant glioma cells.

Considering the tumor-promoting role of Beclin1 in melanomas, scientists have found that targeting this Beclin1 leads to increased infiltration of functional NK cells into the melanoma tumors, which ultimately leads to inhibition of tumor growth by promoting tumor shrinkage and, hence, suggesting an effective immunotherapy-based treatment for the melanomas and culminates to increased survival of cancer patients (Mgrditchian *et al.* 2017).

Consistent with this finding, a similar induced autophagic effect has been reported upon treatment with a chemotherapeutic drug fasudil in the oesophageal squamous cell lines (Xie *et al.* 2018). Together, these studies suggest the upregulated autophagy as a survival mechanism up-taken by tumor cells to maintain excessive growth. In another cellular setting, endostatin; a potent anti-angiogenesis agent-induced autophagic cell death, has been observed in human endothelial cells with the characteristic features of autophagic vacuoles (Chau *et al.* 2003), suggesting that these therapeutic agents trigger the tumor microenvironment for autophagic cell death, which in turn, become a process by which cancer cells meet high energy requirements for their growth and survival.

Furthermore, considering one of the mechanisms behind the highly upregulated autophagy-driven tumor promoter activity in cancer cells, it has been found in the cellular

setting of myelomas that the transcription factor IRF4-mediated upregulation of a heterodimeric protease caspase-10/cFLIPL results in cleavage of a Bcl-2-associated protein named as BCLAF1 which ultimately leads to uncontrolled autophagy and this autophagy is being used by the myeloma cells as a mean of fulfilling their exaggerated growth requirement (Lamy *et al.* 2013). This finding supports the probable mechanism behind the tumor promoter activity of the Beclin1-mediated autophagy. In breast cancer, being the second most common cause of death due to cancer among females, the most effective drug for estrogen receptor (ER)-positive breast cancer, tamoxifen, shows limited efficacy due to drug resistance in these cancer cells. In search of the cause of this drug resistance, Beclin1 and human epidermal growth factor receptor 2 (HER2) are upregulated and contribute to tumor progression. The elevated level of Beclin1 limits the overall survival of ER-positive breast cancer patients, and later, upon silencing, Beclin1 abrogates its negative impact on the survival of breast cancer patients treated with tamoxifen drug (Gu *et al.* 2016). Another similar study reported the upregulation of Beclin1 in the tamoxifen-resistance human breast cancer cell lines but with a synergistic increase in the expression of lactate dehydrogenase A (LDHA) along with the Beclin1. This synergistic expression results in an increase in the expression of anti-apoptotic protein Bcl-2 and hence contributes to the apoptosis resistance in these cells. Further, pharmacological/genetic inhibition of LDHA reactivates apoptosis and sensitizes the ER-positive breast cancer cells (Das *et al.* 2019). This study suggests the concurrent role of Beclin1, LDHA, and Bcl-2 in the tumorigenesis of ER-positive breast cells and, subsequently, switching from the tumorigenic state to the apoptotic state. Together, these findings support the drug resistance-driven tumor-promoting role of Beclin1 in breast cancer.

Another study reveals a high expression level of Beclin1 in intrahepatic cholangiocarcinoma (ICC), leading to a poor prognosis in ICC patients (Bi *et al.* 2019). This finding supports the tumor-promoting activity shown by Beclin1 in ICC tumor tissues.

Moreover, upregulated expression of the Beclin1 is also observed in hepatocellular carcinoma. In these malignant cells, Beclin1 is associated with another protein 14-3-3ζ under hypoxia (2% oxygen) condition, leading to enhanced autophagy due to chemo-resistance, suggesting the tumor-promoting role of Beclin1 in primary liver cancer (Tang *et al.* 2020). Similar effects of Beclin1 have been shown in other forms of cancer, such as ovarian carcinogenesis (Zhao *et al.* 2014) and endometrial adenocarcinomas (Giatromanolaki *et al.* 2011). Furthermore, in non-small cell lung cancer (NSCLC), the overexpression of Beclin1 has been correlated with enhanced migration of NSCLC cells, and the Beclin1 upon knockdown results in reduced migratory ability. Additionally, it is found that the migration in the NSCLC cells is due to the Beclin1-Vimentin interaction. This study supports the tumor-promoting role of Beclin1 in NSCLC cells (Cheng *et al.* 2019). In urothelial carcinoma cells (UCCs), the chemotherapeutic drug cisplatin-induced autophagy has been observed with marked elevation in the expression of several autophagy-related proteins of the VPS34-Beclin1 complex. Further, upon administration of the autophagy inhibitors chloroquine or 3-MA, these cancer cells are found to be sensitized to cisplatin-mediated cytotoxicity in the UCCs (Schlütermann *et al.* 2018). This finding supports the tumor-promoting role of Beclin1, which is found to be dominating in the cisplatin-resistance in bladder cancer. In another study on rectal carcinoma tissues, in a stressed microenvironment, the level of Beclin1 is overexpressed, which results in cancer cell survival and tumor progression (Zaanan *et al.* 2015). Consistent with this finding, in the preclinical trials, the human rectal carcinoma tissues become sensitive to fluorouracil (5-FU) upon siRNA-mediated knockdown of beclin1 (Sui *et al.* 2014). Moreover, it is well known that the existing cancer therapies induce DNA double-strand breaks (DSBs) that activate DNA repair mechanisms to restore genomic integrity. In line with this, the study reported that radiation-treated colorectal cancer cell lines show induced expression of Beclin1, which confers resistance to DNA DSBs. Later, the

siRNA-mediated knockdown of beclin1 in irradiated colorectal cancer cells increases in the radiation, and 5-FU-induced DNA double-strand breaks and promotes apoptotic cell death (Park *et al.* 2014). In the same cellular setting of colorectal cancer cells, the anti-cancer drug bevacizumab-induced resistance raises a prime concern. The reason behind this drug resistance is due to upregulation in the Beclin1-mediated autophagy in these cancer cells. Further, the chloroquine or siRNA-mediated inhibition of autophagy promoted bevacizumab-dependent sensitivity as well as apoptosis in colorectal cancer cells (Zhao *et al.* 2018). Additionally, a very recent study reveals the association of beclin1 mRNA via its 3'-UTR region with a heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) in the pathogenesis of colorectal cancer cells. This study also finds the upregulated expression of both hnRNPA1 and beclin1 mRNA in the colorectal cancer cells, and further, upon knockdown of hnRNPA1 results in a decline in the expression of beclin1 mRNA, suggesting their positive correlation in the pathogenesis of colorectal cancer (Ji *et al.* 2019). Altogether, these findings indicate the tumor-promoting role of Beclin1 during drug resistance in cancer cells. In a separate finding, it has been found that in the stressed condition (nutrient deprivation for 4 hours), Beclin1 shows tumor promoter activity (hence, induced autophagy) upon dissociating with the anti-apoptotic protein Bcl-2. This dissociation of the Beclin1-Bcl-2 complex occurs due to the small amount of JNK1-mediated Bcl-2 phosphorylation. It is essential to mention that this small amount of Bcl-2 phosphorylation leads only to disrupt the Beclin1-Bcl-2 complex and dissociate the Bcl-2, but not to disrupt the Bcl-2-BAX complex, resulting in induced autophagy and reduced apoptosis (Wei *et al.* 2008a). This study suggests the tumor promoter behavior of Beclin1, which is dependent on the anti-apoptotic protein Bcl-2 (Figure 1.3). Altogether, these observations reveal the tumor promoter role of Beclin1 as a key player involved in the induced autophagy in the stressed environment in the various forms of cancer.

Taken together, these studies support the tumor-promoting behavior of autophagy. In these cancer cells, the progressive stress level compels the process of upregulated autophagic cell death to fulfill the uplifted growth requirements. The upregulated autophagic cell death promotes metastasis, invasion, and aggressiveness in these cancer cells. The enhanced autophagy also renders the cancer cell resistant to chemotherapeutic drugs and prevents apoptotic cell death.

1.3.3 Autophagy in Stress (Beyond Threshold Value), Switched to Apoptotic Cell Death

It is difficult to delineate the accurate distinction between the apoptotic and autophagic forms of cell death as these two forms of death are not mutually exclusive processes; rather, the extreme upregulated forms of cell death, which depend on type and level of stress which ultimately culminate to shifting of the cell's response from survival-based adaptive autophagy to the apoptotic form of cell death (Gewirtz 2020) (Figure 1.3). Genetic or pharmacological inhibition of autophagy has been shown to cause a significant increase in the oncosuppressive activity of several promising anti-cancer agents. Growing evidence in the literature highlights this aspect of autophagy. In this line, inhibition of Beclin1 by overexpressing miR-30a-5p results in an increase in the apoptosis and, hence, in combating the drug resistance in the etoposide/cisplatin-treated human small cell lung cancer (SCLC) cells (Yang *et al.* 2017). Furthermore, a study on oesophageal squamous cell lines suggests the fasudil (a chemotherapeutic drug) treatment-induced autophagy gets switched to apoptosis upon knockdown of the *beclin1* gene and administration of the pharmacologic agent chloroquine (Xie *et al.* 2018). Another study on myeloma cells also indicates the hyperactive autophagy-driven cell death in myeloma cells. In this finding, they have reported that upon inhibiting the caspase-10 using a broad-spectrum caspase inhibitor, a carboxy-terminal phenoxy group conjugated to the amino acids valine and aspartate (Q-VD-OPh) results in increased association of BCLAF1 and Bcl-2 and thereby, decreased association of Beclin1 and Bcl-2.

Table 1.2. Pre-clinical and clinical evidence of aberrant expression of Beclin1-mediated autophagy in several cancers.

Disorder	Pharmacological Administration	Effect on Autophagy	Effect on Apoptosis	Genetic Inhibition of Autophagy/Apoptosis	Overall Effect after Genetic/Pharmacological Inhibition of Autophagy/Apoptosis on Disorder	Ref.(s)
Tumor Suppressor Role						
Gliomas	β-asarone	Elevated Beclin1 expression, hence, exerting tumor suppressor role	Inhibited expression of Bcl-2	-	Inhibited cell proliferation; Elevated Beclin1 expression Promotes autophagy	(Wang *et al.* 2018)
Ovarian Carcinoma	Resveratrol	Resveratrol-mediated induction in autophagy	Inhibited cell growth with the characteristic feature of apoptosis	-	Resveratrol-treated tumor suppressor behavior of Beclin-mediated autophagy	(Opipari *et al.* 2004)
Precursor B-cell Lymphoma	Lipopolysaccharide-induced septic shock	Inhibition of myeloid-specific Beclin1	-	-	Tumor suppressor role of Beclin1-mediated autophagy	(Tan *et al.* 2019)
Tumor Promoter Role						
Colon cancer	Rapamycin	Activated autophagy, hence, exerting tumor promoter role	-	siRNA targeting *beclin1*	Suppression of epithelial-to-mesenchymal transition; Inhibited invasiveness of colon cancer cells	(Shen *et al.* 2018)
Colorectal carcinoma	Bevacizumab	Elevated Beclin1 expression, hence, exerting tumor promoter role Bevacizumab-resistance	-	siRNA targeting *beclin1*	Sensitization to Bevacizumab in the cancer cells	(Zhao *et al.* 2018)
ER-positive Breast Cancer	Tamoxifen	Elevated Beclin1 expression, hence, exerting tumor promoter role; Tamoxifen-resistance	-	siRNA targeting *beclin1*	Abrogates the negative impact on the survival of ER-positive breast cancer patients	(Gu *et al.* 2016)
Triple-Negative Breast Cancer	Thymoquinone	Beclin1-mediated proliferation,	-	-	Inhibited expression of Beclin1 and autophagic activity in	(Ünal *et al.* 2020)

(TNBC)		migration, and invasion in TNBC cells, hence, exerting tumor promoter role			thymoquinone-treated TNBC cells	
Urothelial Carcinoma	Cisplatin	Elevated Beclin1 expression, hence, exerting tumor promoter role; Cisplatin-resistance	-	-	Chloroquine- or 3-MA-mediated sensitization to Cisplatin-mediated cytotoxicity in these cells	(Schlütermann *et al.* 2018)
Switching from Autophagy to Apoptosis						
Breast cancer	Paclitaxel	Induced autophagy	-	siRNA targeting *beclin1*	Switching from autophagy to apoptosis	(Wu *et al.* 2019)
Chronic Myeloid Leukemia (CML)	Imatinib	Upregulated expression of Beclin1; Induced autophagy	-	-	Spautin-1-mediated downregulation of Beclin1; Reduced autophagy; Decreased Bcl-2 expression; Switching from autophagy to apoptosis	(Shao *et al.* 2014)
Colorectal cancer	Mitomycin C + Bortezomib	Dephosphorylation-mediated increased cleavage of Beclin1, hence, disruption of the R-BiP/Beclin-1/p62 complex	Induced apoptosis	-	Switching from autophagy to apoptosis	(Song *et al.* 2018)
Gastrointestinal Stromal tumors	Imatinib	Upregulated expression of Beclin1, hence, exerting tumor promoter role; Induced autophagy; Resistance to imatinib	-	miR-30a-mediated autophagy inhibition	Downregulation of Beclin1; Increase in apoptosis; Switching from autophagy to apoptosis; Re-sensitization to Imatinib	(Chen *et al.* 2020)
Hypertrophic scar fibroblasts	Ursolic Acid (UA)	Induced autophagy; Upregulated expression of Beclin1	Induced apoptosis; Deregulated expression of Bcl-2	siRNA targeting *beclin1*	Promotes UA-induced apoptosis upon siRNA-mediated knockdown of *beclin1*	(Cao *et al.* 2018)

Hypopharyngeal squamous cell carcinoma	Cisplatin	Upregulated expression of Beclin1; Induced autophagy; Confer cisplatin-resistance to cancer cells	-	-	Increase in apoptosis in 3-MA-treated cells; Decreased expression of Beclin1 and decline in numbers of autophagic vacuoles in 3-MA-treated cells; Re-sensitization of cisplatin-resistant cells; Switching from autophagy to apoptosis	(Zhang *et al.* 2020)
Oesophageal squamous carcinoma	Fasudil + Chloroquine	Induced autophagy	-	siRNA targeting *beclin1*	Switching from autophagy to apoptosis	(Xie *et al.* 2018)
Oral squamous cell carcinoma	Cisplatin	Upregulated expression of Beclin1; Confer cisplatin-resistance to cancer cells	-	miR-30a mimics-mediated autophagy inhibition	Decreased Beclin1 expression; Decreased Bcl-2 expression; Re-sensitization of cisplatin-resistant cells	(Kulkarni *et al.* 2020)
Osteosarcoma	Doxorubicin	Elevated Beclin1 expression, hence, exerting tumor promoter role; Upregulated autophagy in Doxorubicin-resistant cells	-	Overexpression of miR-30a	Suppressed Beclin1 expression; Reduced autophagy; Reduced chemoresistance; Induced apoptosis	(Xu *et al.* 2016)
Ovarian cancer	S1 (a BH3 mimetic)	Disrupts Beclin1 and Bcl-2 interaction; Induced autophagy	Inhibition of Bcl-2; Induced Apoptosis	-	S1-induced apoptosis is enhanced upon 3-MA and chloroquine treatment; S1-induced overactivation of Caspases; Caspase-mediated cleavage of Beclin1; Sensitization of cancer cells to apoptosis	(Li *et al.* 2015)
Small cell lung cancer	Etoposide+ Cisplatin	Upregulated expression of Beclin1	Increase in apoptosis	miR-30a -5p- mediated autophagy inhibition	Re-sensitizing the drug-resistant cancer cells to chemotherapy	(Yang *et al.* 2017)

The declined interaction between Beclin1 and Bcl-2 results in hyperactive autophagic cell death in myeloma cells (Lamy *et al.* 2013). However, it is mere speculation that down the line, possibly, there may be caspase-8-mediated cleavage of Beclin1 would take place, and that would lead to translocation of cleaved Beclin1 into mitochondria and ultimately mitochondrial outer membrane permeabilization-dependent release of cytochrome c. These cellular events may culminate in the myeloma cells entering into the process of apoptosis. This speculation may support the switching process from autophagy to apoptosis. Initially, Beclin1 was discovered as a Bcl-2-interacting protein (Liang *et al.* 1998), and this interaction between Beclin1 and Bcl-2 has been known to form an established molecular connection between autophagy and apoptosis. It is also reported that both Beclin1 and Bcl-2 play a vital role in cancer. The BH3 domain of Beclin1 has been found to interact with the Bcl-2, and the interrupted interaction of these two molecules may lead to dysregulated autophagy and apoptosis. Experimentally, it has been found that in the stressed condition (nutrient deprivation for 16 hours), a Beclin1-dependent transition from autophagy to apoptosis takes place. During this switch, there is disruption of the Beclin1-Bcl-2 complex, which is due to a maximum amount of JNK1-mediated Bcl-2 phosphorylation. This Bcl-2 phosphorylation leads to dissociation of the Bcl-2 from the Beclin1-Bcl-2 complex as well as from the Bcl-2-BAX complex. The detection of active Caspase-3 accompanies this incident in the cell and indicates the initiation of apoptosis (Wei *et al.* 2008a). This suggests the key regulators of these two forms of the death-mediated switch from autophagy to apoptosis in the stressed microenvironment. In the BT-474 breast cancer cell line (*in vitro*) as well as in the xenograft model (*in vivo*), a chemotherapeutic drug paclitaxel-induced transition from autophagy to apoptosis has been observed in a dose- and time-dependent manner upon knockdown of beclin1 (Wu *et al.* 2019).

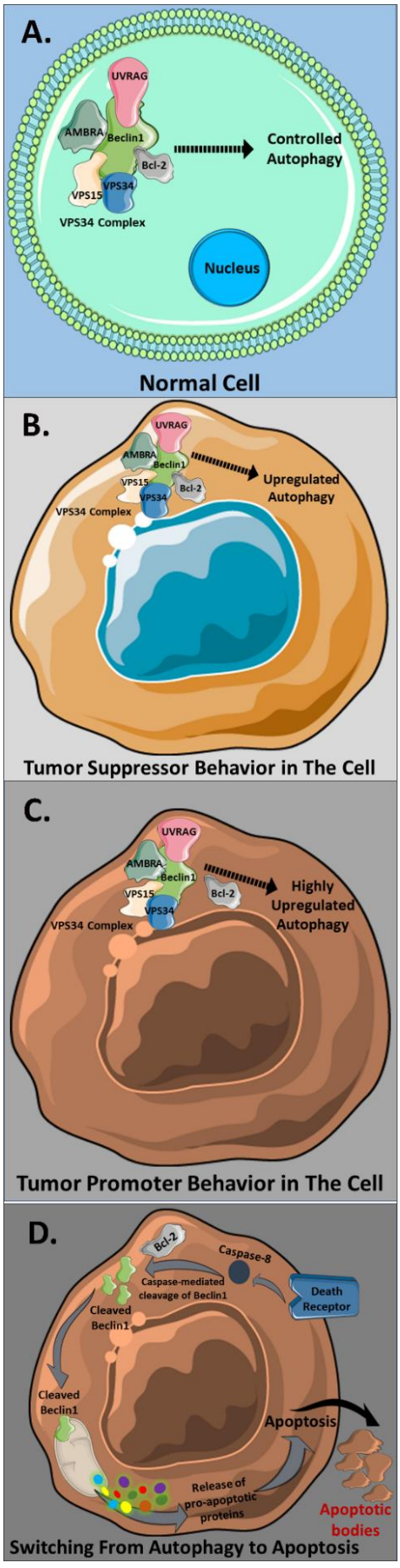

Figure 1.3. The cross-talk between autophagy and apoptosis. (A) In normal conditions, the cell utilizes autophagy to recycle the metabolites and nutrients from the damaged organelles and long-lived proteins. This recycling mechanism can be considered 'autophagic cell death' or 'adaptive autophagy' and a controlled process. During this controlled scenario of autophagic cell death, the anti-apoptotic protein Bcl-2 (a key regulator of apoptosis) is bound with the BH3 domain of Beclin1. Although this binding of Bcl-2 to Beclin1 is anti-autophagic and occurs to prevent the erroneous activation of autophagy, the overall process of autophagy is controlled. **(B)** when the cell gets exposed to physiological stress (below the threshold value), then the process of adaptive autophagy becomes upregulated. During this phase, stress-induced activation of JNK1 occurs, which causes a minimal level of JNK1-mediated Bcl-2 phosphorylation, and most of the Bcl-2 are bound to Beclin1, and the process of autophagy is still running in a controlled manner but upregulated. This upregulation arises to circumvent the physiological stress, which might be tumorigenic and, therefore, acts as a tumor suppressor facet of autophagy. **(C)** When the stress prolongs and gets more intensified and leads to tumorigenesis, the process of autophagy becomes a means to resist stress. During this stage, the cell's tumor suppressive activity is hijacked by the tumorigenic state of the cell to meet the tumor cell's exaggerated growth requirements. Therefore, now the same process of upregulated autophagy, which was earlier regarded to show tumor suppressor behavior, indicates the tumor promoter behavior and acts as a barrier against apoptosis. During this phase, the JNK1-mediated Bcl-2 phosphorylation is increased to an extent, leading to disruption of the Beclin1-Bcl-2 complex and, hence, dissociation of Bcl-2. Altogether, these processes culminate in highly upregulated autophagy. **(D)** In this scenario, simultaneously, the stress level reaches beyond the threshold value, and the process of highly upregulated autophagy starts declining and transitioning from autophagy to apoptosis. It is hard to delineate the process of transition from autophagy to apoptosis. During this phase, the JNK1-mediated Bcl-2 phosphorylation is maximal, leading to disruption of the Beclin1-Bcl-2 complex and the Bcl-2-BAX complex and, hence, dissociation of Bcl-2. This favors the pro-apoptotic proteins to initiate apoptosis. Additionally, the elevated level of Beclin1 also declined due to caspase-mediated cleavage of Beclin1. Altogether, a 'toggle switch' becomes ON in the cell, facilitating the transition from autophagy to apoptosis.

Therefore, this suggests the switching of tumorigenic state (due to upregulated Beclin1) to caspase-dependent apoptosis in breast cancer cell lines. Furthermore, in a separate finding, it has been found that the Imatinib-associated resistance (due to induced autophagy and, as a result, elevated Beclin1 expression) in chronic myeloid leukemia (CML) cells may be circumvented by the use of spautin-1 (specific and potent autophagy inhibitor-1) in combination with imatinib. These drugs synergistically cause the transition from autophagy to apoptosis with a marked downregulation in the Beclin1 and the Bcl-2 levels in the CML cells (Shao *et al.* 2014). Therefore, the interaction between Beclin1 and Bcl-2 is crucial to unravel as far as cancer is concerned. So, in this direction, in our very recent computational work, we have attempted blocking the interaction of the BH3 domain of Beclin1 and Bcl-2 using FDA-approved drugs, and based on different investigating parameters, we have anticipated that the top three FDA-approved drugs viz. ponatinib, simeprevir and nilotinib can be the potent regimen candidates, however, once the adequate experimental pieces of evidence can be achieved, the drug ponatinib may be used as a remarkable drug for further research in the domain of autophagy-apoptosis cross-talk (Prerna and Dubey 2021). The mechanism of inhibition of autophagy and activation of apoptosis by using these drugs is still elusive; however, together with our investigative study on targeting the protein-protein interactions for inhibiting autophagy using FDA-approved drugs and thereby activating apoptosis, it may become a novel strategy that may give an insight into the protein dynamics and play a crucial role in the drug discovery.

In a recent study, scientists have observed the switch from highly induced autophagy to apoptosis in the human synovial sarcoma cells. They have overexpressed the Beclin1 in the SW982 human synovial sarcoma cells and observed the cell viability, and found that the sarcoma cells with overexpressed Beclin1 resulted in poor cell viability with induced apoptosis with the marked presence of a decline in the expression level of the anti-apoptotic

protein Bcl-2 and an increase in the level of cleaved initiator caspase; the caspase-9 and cleaved executioner caspase; the caspase-3, which suggests the role of upregulated autophagy in promoting apoptosis in the tumor cells (Zhu *et al.* 2018). Similarly, a study on the effect of over-expression of Beclin1 in gastric cancer has been reported wherein they have reported that the over-expression of the protein Beclin1 promotes apoptosis, probably due to the mechanism where over-expression of Beclin1 leads to inhibition of the expression of an anti-apoptotic protein Bcl-xL which ultimately triggers the apoptotic pathway of cell death, in the MKN-45 gastric cancer cell line which culminates to reduced cancer cell migration (Wang *et al.* 2017b). In another cellular setting of gastrointestinal stromal tumors, imatinib-induced autophagy contributes to tumor promotion and, ultimately, to chemoresistance. To circumvent this drug resistance, treatment of microRNA-30a results in the downregulation of beclin1 and sensitization of these cancer cells to imatinib and subsequently in an increase in the apoptosis of these cells (Chen *et al.* 2020). Similar results have been reported in miR-30d-transfected human colon cancer cell lines (Zhang *et al.* 2017). Therefore, these studies support the microRNA-driven transition from the tumor-promoting facet of Beclin1-mediated autophagy to apoptosis in the stressed microenvironment. Similarly, a study on the expression of Beclin1 in hepatocellular carcinoma reveals that in a prolonged stressed microenvironment, the tumor cells show induced expression of Beclin1 along with overexpression of a pro-apoptotic protein Bax and a significant decrease in the expression of anti-apoptotic protein Bcl-2, which together, promote the autophagic state of the hepatocellular carcinomas to apoptosis and lead to inhibited cell proliferation (Qiu *et al.* 2014). In some cellular settings, a caspase-mediated transition from autophagy to apoptosis has been reported. It has been found that the Bax disrupts the interaction between Beclin1 and Bcl-xL, and thereby the beclin1 at D149 position gets cleaved by the caspase where they used sustained growth factor-deprived Ba/F3 cells and observed caspase-dependent cleavage of

Beclin1 at D133 and D149 (caspase-mediated cleavage sites on the Beclin1) leading to a reduced level of autophagy and induced level of apoptosis in these Ba/F3 cells (Wirawan *et al.* 2010).

These observations together may suggest the probable demarcation of transition from the tumor promoter facet of autophagy to apoptotic cell death in the cancer cells. Hence, inhibiting autophagy in tumor-promoting cells by targeting Beclin1 through external means promotes cell death. Therefore, Beclin1 may be utilized as a potential biomarker for enhancing the effect of chemoradiation on cancer cells.

1.4 Autophagy in other pathophysiologies

The aberrant expression of Beclin1 is evident in cancer; however, its aggressiveness has also been witnessed in several other pathophysiologies, viz. neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease, and Huntington's disease, cardiac disorders, inflammatory, and viral diseases. The dual behavior of autophagy as both cytoprotective and cytotoxic can be observed in several growing studies on different pathophysiologies. In this section, we have tried to categorize these two facets of autophagy for individual pathophysiology (Table 1.3).

1.4.1 Autophagy in Neural Disorders

Upregulated Autophagy as a Cytoprotective Mechanism: In Alzheimer's disease, Beclin1-mediated autophagy provides cytoprotective behavior by regulating amyloid precursor protein processing. The reduced level of Beclin1 results in improper autophagic degradation and accumulation of protein aggregates, which are the most common characteristic features for developing Alzheimer's disease. In a recent study, the Caspase 3-mediated cleavage of Beclin1 and, subsequently, subcellular redistribution of its cleaved fragments have been observed, resulting in a reduction in the Beclin1 full-length protein and thereby culminating in defective protein clearance in cells with AD-like neuronal injury (Wang *et al.* 2017a). In

neurodegenerative diseases, the polyglutamine tracts have been known to exert toxic effects, and recently, Ashkenazi et al. reported the correlation of polyglutamine tracts-containing proteins with autophagy. In this correlation, the polyglutamine tract-containing proteins interact with Beclin1, and the autophagy-mediated clearance of aggregates occurs. However, the mutation in the polyglutamine tract-containing proteins competes with wild-type polyglutamine tract-containing proteins for the interaction with Beclin1 and causes abrogation of autophagy and hence exertion of deleterious effects on the cell (Ashkenazi *et al.* 2017). Moreover, the cytoprotective behavior of autophagy is also observed during Beclin1-mediated endolysosomal and autophagic degradation of plasma membrane-associated amyloid β precursor protein in Alzheimer's disease (Swaminathan *et al.* 2016).

Very recently, the probable mechanism behind the cytoprotective behavior of Beclin1 has been highlighted. In this study, upon glucose deprivation and hypoxia (acting as stressors), the increased production of reactive oxygen species (ROS) results in the induction of Beclin1-mediated autophagy through a specific axis, referred to as ataxia-telangiectasia mutated (ATM)-cell cycle checkpoint kinase 2 (CHK2)-Beclin1 axis. In this axis, the CHK2 binds to the CCD and ECD domain of Beclin1 and phosphorylates it at Ser90/Ser93. This CHK2-mediated phosphorylation impairs the interaction between Beclin1 and Bcl-2 and subsequently reduces the ROS level by removing damaged mitochondria and culminating in cell survival (Guo *et al.* 2020). This study supports the cytoprotective behavior of Beclin1 in the ROS-driven cellular stressed microenvironment.

Upregulated Autophagy as a Cytotoxic Mechanism: In the stressed environment, the upregulation in the process of autophagy renders both cytoprotective and cytotoxic activity. Recently, upregulation in autophagy has been observed upon a high concentration of glutamate-triggered cytotoxicity in murine HT22 hippocampal neuronal cells. Moreover, in these stress-triggered cells, Beclin1 gets cleaved explicitly by calpain to generate a C-

terminal fragment (40-kDa) of Beclin1. This cleaved fragment no longer enters the process of autophagy; instead, it promotes cellular senescence-mediated cell death in these oxidative stress-induced neuronal cells (Nguyen *et al.* 2019). This suggests the critical involvement of Beclin1 in switching from the process of autophagy to non-autophagic cell death in the stress-induced cells. Furthermore, some viruses target the key autophagy protein Beclin1 to escape from their autophagic degradation. In this line, one of the viruses is Enterovirus 71 (EV71), which binds to Beclin1 through its conserved evolutionary domain (ECD) and coiled-coiled domain (CCD). This binding promotes the replication of EV71 in human rhabdomyosarcoma cells and human astroglioma cells and causes hand-foot-mouth disease (HFMD), which is prevalent in infants and children and leads to severe neurological disorders and even death in the same age groups (Xiang *et al.* 2020). This study suggests the role of Beclin1 in viral replication that ultimately led to progression in the EV71-associated diseases. These progressive dysfunctionalities have been observed due to autophagic cell death.

1.4.2 Autophagy in Cardiac Disorders

Upregulated Autophagy as a Cytoprotective Mechanism: Several ongoing studies have highlighted the correlation between defective autophagy and cardiopathies, indicating the Beclin1-mediated autophagy as a cytoprotective mechanism to circumvent cardiac disorders. In this line, a recent study has shown the interaction of a novel cardio protector HSPB6 with Beclin1, which aids in cell survival during stress-induced injury. Furthermore, this study has shown that HSPB6-induced overexpression of Beclin1 results in limited binding of Beclin1 to Bcl-2, thereby upregulating the autophagy to prevent stress-induced damage in heart cells (Liu *et al.* 2018a). Myocardial stunning and hibernation are the physiological conditions of adaptation to ischemia, and autophagy has been considered the underlying adaptive mechanism of cardiac myocyte survival during stress. In atherosclerosis, cholesterol influx is the primary cause for its development, and to circumvent this cause, Huang et al. revealed the

interferon-stimulated gene 15 (ISG15)-dependent molecular mechanism behind the Beclin1-mediated autophagy, which leads to cholesterol efflux from the macrophage-derived foam cells and is shown to be a practical approach in the abrogation of development of atherosclerosis (Huang *et al.* 2018).

Extensive research in the past indicates the involvement of excessive autophagy with a marked upregulation in Beclin1 in stressed (due to hypoxia/reoxygenation) human cardiomyocytes. These experiments are performed to find the underlying mechanism behind the myocardial ischemia/reperfusion injury, which is caused by restored blood supply after acute myocardial infarction. This finding indicates the role of excessive autophagy as a cytoprotective mechanism against the apoptotic cell death caused due to myocardial ischemia/reperfusion injury (Shi *et al.* 2019), thereby suggesting the interplay between autophagy and apoptosis in heart diseases. Similar effects of Beclin1-mediated upregulated autophagy have been reported in chronic intermittent hypoxia (CIH)-treated human coronary artery endothelial cells (HCAECs). This beclin1-mediated autophagy is found to be enhanced upon miR-34a-5p-mediated downregulation of Bcl-2. Hence, this study suggests the role of autophagy-apoptosis interplay as a cytoprotective mechanism in stressed cardiomyocytes; thereby, it may become a therapeutic strategy to treat cardiovascular diseases (Lv *et al.* 2019).

Table 1.3. Dual role of Beclin1-mediated autophagy in several pathophysiology.

Disorder	Effect on Autophagy	Effect on Apoptosis	Pharmacological Inhibition of Autophagy/Apoptosis	Genetic Inhibition of Autophagy/Apoptosis	Overall Effect after Genetic/Pharmacological Inhibition of Autophagy/Apoptosis on Disorder	Ref.(s)
Neural Disorders						
Alzheimer's Disease	Beclin1-mediated processing of amyloid precursor protein; Cytoprotective role of Beclin1	-	-	Caspase-3-mediated cleavage of Beclin1	Reduced level of Beclin1; Improper autophagic degradation; Accumulation of protein aggregates	(Wang *et al.* 2017a)
Amyotrophic Lateral Sclerosis (ALS)	Co-expression of Beclin1; Enhanced autophagy	-	-	-	Cytoprotective role of Beclin1; Autophagic degradation of ALS-linked superoxide dismutase 1 (SOD1) mutant	(YM and B 2018)
Hand-Foot-Mouth Disease (HFMD)	Binding of Enterovirus 71 to Beclin1. This binding induces Enterovirus 71 replication in the host cell	-	-	-	Cytotoxic role of Beclin1; Progression in the EV71-associated diseases	(Xiang *et al.* 2020)
Cardiac Disorders						
Dilated Cardiomyopathy	Beclin1 ubiquitination and its proteasomal degradation; Inhibited autophagy	Increased apoptosis	-	-	Cytoprotective role of Beclin1; Heart failure and early death	(Liu *et al.* 2018a)
Diabetic Cardiomyopathy	Upregulated Beclin1 expression, hence, Beclin1 is exerting a cytotoxic role; Increased autophagy	Upregulation of pro-apoptotic protein Caspase 3	Chloroquine-mediated inhibition of autophagy	Genetic depletion of Beclin1	Inhibited the autophagy in high glucose-treated cardiomyocytes; Inhibited the apoptosis in high glucose-treated cardiomyocytes	(Munasinghe *et al.* 2016)
Inflammatory Diseases						
Osteoarthritis	Overexpression of Beclin1; Upregulation in Bcl-2; Downregulation of BAX	Enhanced chondrocytes apoptosis	-	-	Inhibition of apoptosis; Cytoprotective role of Beclin1	(Song *et al.* 2017)

Lymphoid Malignancies	High inflammatory mediator production, hence, hypersensitive neutrophils	-	Adiponectin-mediated inhibition of autophagy	-	Disrupted interaction between the Beclin1 and Bcl-2 and induced Bcl-2 mRNA destabilization; Suppression of inflammatory mediator production; Cytoprotective role of Beclin1	(Pun and Park 2018)
Renal Disorder						
Kidney I/R Injury	Upregulated Beclin1 expression, hence, Beclin1 is exerting a cytotoxic role in this injury; Increased autophagy	Increased apoptosis	-	miR-30a-5p Mimic-mediated inhibition of autophagy	Cytoprotective role of Beclin1; Downregulated Beclin1 expression, hence, decreased autophagy; Decreased apoptosis	(Y *et al.* 2021)
Respiratory Disorder						
Asthma	Dysregulated autophagy; Upregulated Beclin1 expression, hence, exerting the cytotoxic role	-	Chloroquine-mediated inhibition of autophagy	-	Reduced airway inflammation	(McAlinden *et al.* 2019)
Bacterial Infections related Disease						
Intracellular *Listeria monocytogenes* infection	Increased interaction of the E3 ubiquitin ligase NEDD4 with Beclin1, thereby increased stability of Beclin1; Increased autophagy	-	-	-	Cytoprotective role of Beclin1; Killing of intracellular bacterial pathogen	(Pei *et al.* 2017)

Upregulated Autophagy as a Cytotoxic Mechanism

Furthermore, the role of Beclin1 has also been studied to be cytotoxic in the progression of atherosclerosis (AS). In human endothelial cells, during AS development, there is a marked increase in autophagy due to the involvement of oxidized low-density lipoprotein (ox-LDL) has been observed. To decipher the cause of this AS development, scientists found long non-coding RNAs (lncRNAs), metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), and a microRNA, miR-216a-5p, acting as the potential regulators of AS development and progression. During the AS development, the MALAT1 and miR-216a-5p have positive and negative impacts, respectively, over the Beclin1-mediated autophagy and survival of ox-LDL-treated human umbilical vein endothelial cells (HUVECs). Therefore, the cytotoxic role of MALAT1 are to induce the Beclin1-mediated autophagy in the stressed environment (treatment with ox-LDL) in the HUVECs, and to an extent, to antagonize the negative effect of miR-216a-5p, thereby potentiating the AS development and progression in the HUVECs (Wang *et al.* 2019a).

1.4.3 Autophagy in Inflammatory Diseases

Upregulated Autophagy as a Cytoprotective Mechanism: In a recent study, the process of upregulated autophagy has been shown as a probable cytoprotective approach of treatment in osteoarthritis; wherein, the chondrocytes apoptosis has been proved to be a significant implication in cartilage degeneration, which ultimately results in osteoarthritis. In this study, overexpression of Beclin1 is correlated with the upregulation of Bcl-2 and downregulation of BAX; thereby, suggesting the cytoprotective role of Beclin1 in chondrocytes by inhibiting the apoptosis and hence, considering as a novel treatment for osteoarthritis (Song *et al.* 2017). In lymphoid malignancies, deletion of Beclin1 results in hypersensitive neutrophils. Furthermore, a therapeutic agent adiponectin-dependent cytoprotective behavior of Beclin1-mediated autophagy in suppression of inflammatory mediator production is also highlighted,

wherein this adiponectin-mediated suppression results due to disrupted interaction between the Beclin1 and Bcl-2 and induced Bcl-2 mRNA destabilization; thereby, leads to elevated autophagosome formation, which culminates to downregulated inflammatory cytokines production and hence, suggests the potent anti-inflammatory properties of adiponectin (Pun and Park 2018).

Upregulated Autophagy as a Cytotoxic Mechanism: Aberrant involvement of Beclin1-mediated autophagy has been implicated in several inflammatory diseases such as sepsis and acute lung injury. In line with its validation, silencing of Beclin1 in human pulmonary artery endothelial cells results in reduced expression of proinflammatory genes and proinflammatory mediators, which otherwise remain upregulated in the inflammatory diseases (Leonard *et al.* 2019); hence, suggesting the role of Beclin1-mediated upregulated autophagy in delivering cytotoxic effect in inflammatory diseases.

1.4.4 Autophagy in Viral Diseases

Coronaviruses (CoV) use the process of autophagy in an ambiguous way; wherein, these viruses exploit autophagy to increase the viral replication and attenuate autophagy to escape from the autophagy-mediated viral degradation. At the molecular level, the Middle East respiratory syndrome coronavirus (MERS-CoV) is observed to downregulate the expression of Beclin1 during its multiplication. Furthermore, in the stressed microenvironment, the regulation of Beclin1-mediated autophagy depends on a heat shock protein 90 (HSP90) cochaperone FK506 binding protein 51 (FKBP51). In line with this, it is well documented that the expression of Beclin1 is regulated by ubiquitination and phosphorylation, the two particular post-translational modifications; wherein, AKT1-mediated phosphorylation of Beclin1 results in inhibition of autophagy, and E3 ligase (S-phase kinase-associated protein 2 (SKP2))-dependent ubiquitination results in proteasomal degradation of Beclin1. Moreover, the regulation of SKP2 activity depends on

phosphorylation in a heterocomplex involving FKBP51, PH domain leucine-rich repeat protein phosphatase (PHLPP), AKT1, and Beclin1. In this study, scientists have attempted to inhibit the SKP2 and observed the decrease in the degradation of Beclin1, thereby enhancing the autophagy and, subsequently, reduction in the MERS-CoV multiplication (Gassen *et al.* 2019). This study strongly suggests the molecular link between Beclin1-mediated autophagy and viral replication. The pivotal role of Beclin1-mediated autophagy is also highlighted in response to viral infections. This antiviral response is due to RNase L-generated small dsRNAs-mediated switch from autophagy to apoptosis by promoting the caspase 3-mediated cleavage of Beclin1 (Siddiqui *et al.* 2015). This study highlights the mechanism of RNase L-dependent transitional cell death in response to viral infections. Furthermore, the Beclin1-Bcl-2 interaction is also sought to be crucial in hepatitis B virus (HBV) replication as the HBV X protein (HBx) induces autophagy by disrupting JNK signaling-dependent Beclin1-Bcl-2 interaction, thereby suggesting the cytotoxic role of Beclin1 in viral replication (Zhong *et al.* 2017). The interplay between autophagy and apoptosis is not only limited to cancer but also broadens to several pathophysiologies, indicating that dysregulation in these two forms of cell death can have life savior as well as life-threatening effects.

1.5 Scope and Objectives of the current study

Autophagy is a fundamental process that gets activated under normal and stressed conditions to degrade damaged organelles and aggregated proteins to recycle the nutrients and metabolites and thus plays a crucial role in cell physiology. The process of autophagy is interconnected with another form of programmed cell death, apoptosis. These two significant forms of death; autophagy and apoptosis, have long been interrelated in health and diseases.

The wider physiological role of autophagy and apoptosis in development, aging, and normal cellular processes is so far well understood, but there a limited number of studies have been attempted to crack the code of the intricate interplay between these two forms of cell

death in pathophysiology, viz. cancer, neurodegenerative disorders, cardiac disorders, bacterial, viral, and inflammatory diseases. The limited attempt towards unravelling this complex phenomenon is because of dynamic behaviour of this cross talk. In a stressed microenvironment such as nutrient deprivation or therapeutic intervention, autophagy supports the survival of cells and favors the diseased state of the cell. Thus, contrary to apoptosis, autophagy acts in a diversified manner, depending on cellular context and threshold of stress. Despite growing interest and a recent surge in targeting this complex synergy, this cross-talk is still premature and has not been unraveled completely and only provided insights on some of its key regulators; one of them is Beclin1. This autophagic protein Beclin1 shows stress-dependent ambiguity in several pathophysiologies. In cancer, as the stress progresses in the cell, the behavior of Beclin1-mediated autophagy shows hierarchical fashion, i.e., it changes from tumor suppressor to tumor promoter behavior and is hence associated with chemotherapeutic resistance. Further in this hierarchy, emerging shreds of evidence also support the interplay (between autophagy and apoptosis)-mediated cell death in the tumorigenic microenvironment, but such evidences will need further investigations to be used as a target synergistic pathway in exploring novel chemotherapeutic avenues. With this background, we are presenting the critical objectives of the study as follows:

- Beclin-1 and Bcl-2 selected as targets for deciphering the cross-talk between autophagy and apoptosis using *in silico* approach.
- An *in silico* approach is used for repurposing the FDA-approved drugs against the selected target ATG4A for identifying autophagy inhibitors.
- *In vitro* investigations on Ponatinib as a disrupter for Beclin-1 & Bcl-2 and its effect on the cross-talk using various regulators of autophagy and apoptosis.

Moreover, future studies need to provide more specific functions of Beclin1 and its association with regulators of apoptosis to dissect the functional aspects of this cross-talk in cancer and other diseases.