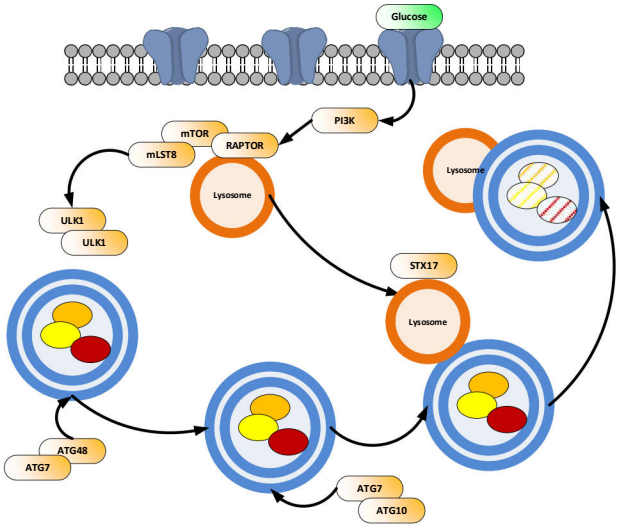

CELL DEATH AND CANCER

September 2024

ABSTRACT

There are multiple types of cell death. Autophagy and Apoptosis are two of the most significant. Each has links to cancers. This Note examines these areas. This Note also supports one we have prepared on macrophages and cancer.

Terrence McGarty

TLC 208

NOTICE

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Our approach herein is to take elements of what is recent in the literature focused on a specific topic and attempt to develop a tapestry image of these connectable elements. We do not necessarily provide any new or fundamental results but merely attempt to assemble elements in a systematic and holistic manner.

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Access to prior Technical Notes can be made via Research Gate,
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Contents

1 INTRODUCTION

Autophagy is the process whereby a cell cleans up the "stuff" left behind by many processes. However autophagy is also involved in many cancers and can be a target for a variety of therapeutics. Moreover autophagy sends out parcels of cleaned up "stuff" which can themselves be either diagnostic or prognostic. We examine some of these issues herein.

However, autophagy can be a benefit and a threat. Autophagy "cleans" up the "stuff" in a cell so that in most cases it can be recycled and reused. However the risk is that if the autophagy takes up key protective proteins thus reducing their efficacy and pays no attention to bad proteins which are now controlling the cell. That is we know that cancer cells have aberrant proteins. We all too often ascribe this to some genetic breakdown. What if, instead, it is the clean-up mechanism of autophagy. Namely every time a p53 gene creates a protein that the specific autophagy targets it for removal. Then we have a cell with no control.

Thus the questions we should be asking regarding autophagy are:

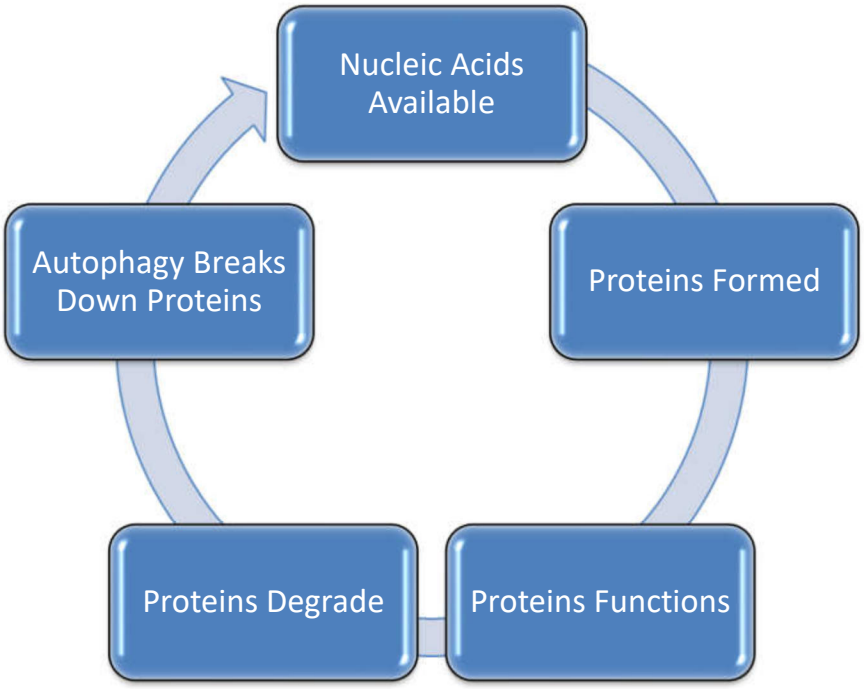
1. What are the dynamics of the process?

2. What makes a protein a target? How does the autophagy process recognize it and why?

3. How do some proteins manage to avoid autophagy?

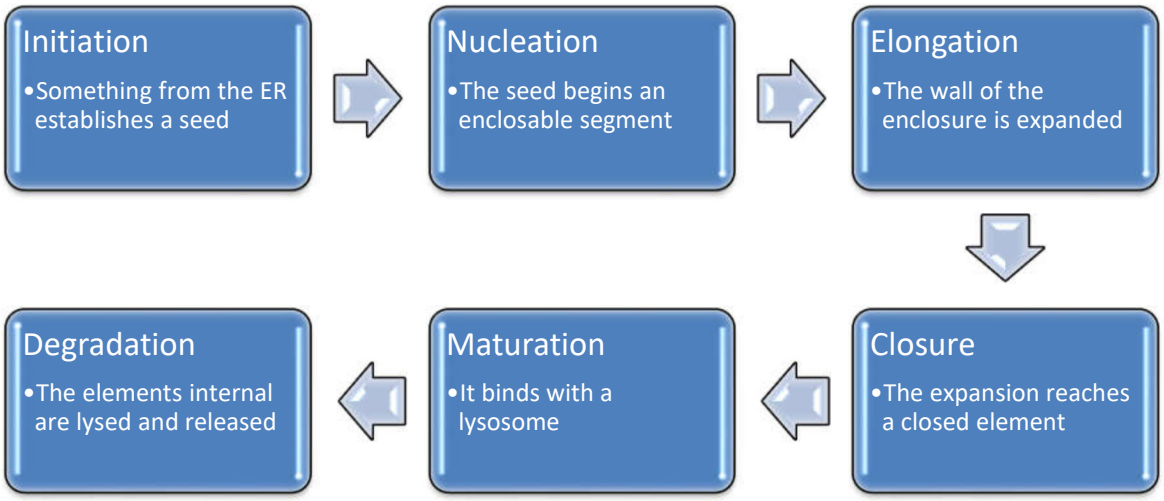
4. How could we envision a method to control or remedy a process?

We can envision the autophagy process as shown simply below. It functions of collecting and degrading old proteins, as an example, returning them to nucleic acids, to be used again. It is an internal process of a cell to maintain homeostasis. However like all cell processes it may go awry, and no longer function efficaciously but be harmful.

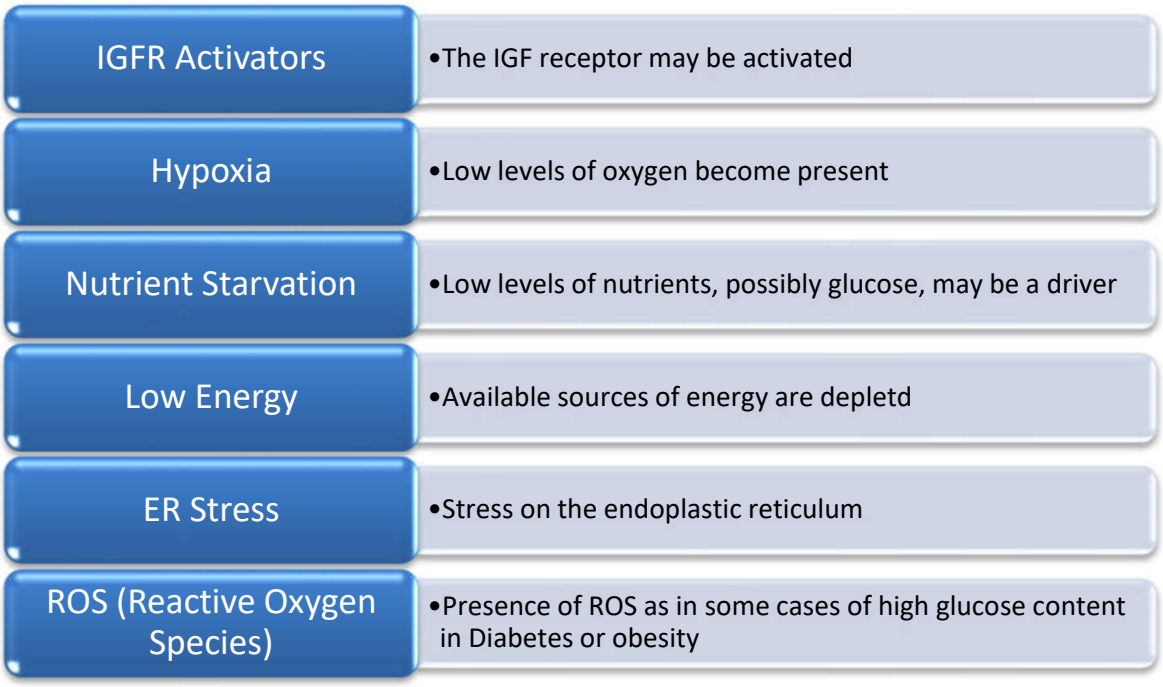


Unlike most papers in the field we do not intend to introduce new ideas or findings but we attempt to concentrate on the above questions.

From a sequential perspective autophagy as per Kang et al progresses as follows:



The initiators are as shown below:



As we shall note herein, the drivers do not seem to include aberrant protein formations[1]. Perhaps the results of such malformations may be drivers for the drivers shown above.

As Kang et al note:

There are at least three different types of autophagy described and possibly more. These autophagy types include macro autophagy (hereafter referred to as autophagy), micro autophagy and chaperone mediated autophagy. The initial step of autophagy is the surrounding and sequestering of cytoplasmic organelles and proteins within an isolation membrane (phagophore). Potential sources for the membrane to generate the phagophore include the Golgi complex, endosomes, the endoplasmic reticulum (ER), mitochondria and the plasma membrane.

The nascent membranes are fused at their edges to form double-membrane vesicles, called autophagosomes. Autophagosomes undergo a stepwise maturation process, including fusion with acidified endosomal and/or lysosomal vesicles, eventually leading to the delivery of cytoplasmic contents to lysosomal components, where they fuse, then degrade and are recycled.

One of the issues that we seem to be lacking insight on, is in the case of autophagy in cancer, either as cause or result, what process leads to the selection of what is to be lysed. We have great insight to the process but little to none as to the initial selection. That will be a critical factor.

[1] See https://www.cancer.gov/publications/dictionaries/cancer-terms/def/igfr *A protein found on the surface of some types of cells that binds to insulin-like growth factor (IGF). This causes the cells to grow and divide. IGFR is found at high levels on the surface of several types of cancer cells, which causes these cells to grow rapidly in the presence of IGF. Also called insulin-like growth factor receptor.*

From a recent paper by Mulcahy et al we have[2]:

Autophagy is a mechanism by which cellular material is delivered to lysosomes for degradation, leading to the basal turnover of cell components and providing energy and macromolecular precursors. Autophagy has opposing, context-dependent roles in cancer, and interventions to both stimulate and inhibit autophagy have been proposed as cancer therapies. This has led to the therapeutic targeting of autophagy in cancer to be sometimes viewed as controversial. ... we suggest a way forwards for the effective targeting of autophagy by understanding the context-dependent roles of autophagy and by capitalizing on modern approaches to clinical trial design.

We shall not focus in detail on their suggestions but try to examine autophagy in general so as to better understand the process.

Yoshinori Ohsumi received the Nobel Prize in Physiology or Medicine in 2016 for his work on autophagy. He spent decades trying to understand the process and its implications. In his presentation he noted[3]:

Life is in an equilibrium state between synthesis and degradation of proteins: replacement of most proteins every 3 months "difference between organisms and machine"

Recycling is essential for life: important ability for survival against starvation critical selection factor in evolution.

To Ohsumi the process of autophagy was one of regeneration not just simple housekeeping. However we know that cells operate as a complex set of internal mechanisms as well as responding to external activations. Furthermore cells send out in exosomes "messages" which in turn may control the behavior of other cells. Autophagy is a process that appears to be very much in the middle of these communications links. It is a transformative process, transforming putative signalling molecules to other putative signalling molecules.

Autophagy appears not to be a simple cleaning up system but a complex element in an ever more complex control system for cellular dynamics. Viewed in this manner we extend what Ohsumi understood to the broader understanding of malignancy control.

As Sengupta et al note in examining mTOR:

Autophagy is a recycling process through which cells liberate intracellular stores of nutrients by degrading cytoplasmic proteins and organelles in lysosomes. In mammalian cells the primary form of autophagy is macroautophagy (referred to from now on as autophagy) and requires the formation of double-membrane autophagosomes that sequester cytoplasmic components and

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[2] **Targeting autophagy in cancer**, Jean M. Mulcahy Levy, Christina G. Towers & Andrew Thorburn Affiliations I Corresponding author, Nature Reviews Cancer Y7, 528-542 (2017) I doi:10.1038/nrc.2017.53, Published online 28 July 2017

[3] https://www.nobelprize.org/prizes/medicine/2016/summary/ and https://www.nobelprize.org/prizes/medicine/2016/ohsumi/auto-biography/

then fuse with lysosomes. A major regulator of autophagy is mTORC1, which in the presence of nutrients and growth factors strongly inhibits the initiation of autophagy.

Autophagy is upregulated during periods of starvation or growth factor withdrawal, as well as in response to oxidative stress, infection, or the accumulation of protein aggregates. While mTORC1 inhibition triggers autophagy, the release of amino acids from autophagic protein degradation eventually leads to the reactivation of mTORC1, which in turn restores the cellular lysosomal population.

Directly downstream of mTORC1 are numerous proteins that are required for the execution of the autophagic program, including the serine/threonine kinase Atg1/ULK, which plays a key role in the formation of the preautophagosome . ULK1 forms a complex with Atg13 and FIP200, which promote ULK1 kinase activity and localization to the preautophagosome.

mTORC1 phosphorylates ULK1 and Atg13, moderately reducing ULK1 kinase activity but not affecting its association with Atg13 and FIP200. Reports conflict about whether mTORC1 binds to the complex under nutrient-replete conditions, and more evidence is needed to determine the role mTORC1 phosphorylation of ULK1 plays in its subcellular localization and interaction with other autophagy proteins. As a result, it is too early to know whether these phosphorylation events fully explain the control of autophagy by mTORC1. Interfering with the ability of cells to undergo autophagy within an intact animal produces a range of phenotypes that underscore the importance of autophagy not only as an adaptive response to nutrient stress, but also in general cell and tissue housekeeping.

For example, mice lacking Atg5, which is required for autophagosome formation, are born at mendelian ratios, but die within 1 day of delivery because they are unable to mobilize the energy and nutrient stores they require to survive the pre-suckling period. Mice depleted of Atg5 in just neural cells exhibit a progressive decline in motor activity that correlates with the buildup of protein aggregates in neurons, indicating that autophagy is essential for the basal clearance of these aggregates and to maintain proper neuronal function in adult animals.

Tissue-specific deletions of additional genes required for autophagy have uncovered roles for autophagy in cardiac contractility, immune cell function, and the liver detoxification of drugs.

2 APOPTOSIS

Apoptosis is a well know process of cell death. It functions by means of activating proteins called caspases. These proteins lie dormant, if you will, in cells until death receptors are activated by ligands, which result in caspases being released and attacking the cell elements, cutting them apart. Apoptosis cuts up the cell elements and packages them away in small elements, thus leaving a dead but compact result of the cutting massacre. The work by Green is an exceptional detailed summary of apoptosis.

2.1 CELL DESTROYERS: CASPASES

Let us begin with understanding the caspases. As Shi notes:

Caspases involved in apoptosis are classified into two groups, the initiator caspases, such as caspase-9 in mammals or its functional ortholog Dronc in Drosophila, and the effector caspases, such as caspases-3 and -7 in mammals and their homolog DrICE in Drosophila.

An initiator caspase invariably contains an extended N-terminal prodomain (>90 amino acids) important for its function, whereas an effector caspase frequently contains 20–30 residues in its prodomain sequence. All caspases are synthesized in cells as catalytically inactive zymogens, and must undergo an activation process. The activation of an effector caspase, such as caspase-3 or -7, is performed by an initiator caspase, such as caspase-9, through an internal cleavage to separate the large and small subunits.

An initiator caspase, however, is autoactivated under apoptotic conditions, a process usually requiring and facilitated by multicomponent complexes. For example, the apoptosome is responsible for the activation of caspase-9…

As McIlwain et al note:

Caspases are a family of genes important for maintaining homeostasis through regulating cell death and inflammation. Here we will attempt to summarize what we currently know about how caspases normally work, and what happens when members of this diverse gene family fail to work correctly.

Caspases are endoproteases that hydrolyze peptide bonds in a reaction that depends on catalytic cysteine residues in the caspase active site and occurs only after certain aspartic acid residues in the substrate.

Although caspase-mediated processing can result in substrate inactivation, it may also generate active signaling molecules that participate in ordered processes such as apoptosis and inflammation.

Accordingly, caspases have been broadly classified by their known roles in apoptosis (caspase-3, -6, -7, -8, and -9 in mammals), and in inflammation (caspase-1, -4, -5, -12 in humans and caspase-1, -11, and -12 in mice).

The functions of caspase-2, -10, and -14 are less easily categorized. Caspases involved in apoptosis have been subclassified by their mechanism of action and are either initiator caspases (caspase- 8 and -9) or executioner caspases (caspase-3, -6, and -7).

Caspases are initially produced as inactive monomeric procaspases that require dimerization and often cleavage for activation. Assembly into dimers is facilitated by various adapter proteins that bind to specific regions in the prodomain of the procaspase. The exact mechanism of assembly depends on the specific adapter involved.

Different caspases have different protein–protein interaction domains in their prodomains, allowing them to complex with different adapters. For example, caspase-1, -2, -4, -5, and -9 contain a caspase recruitment domain (CARD), whereas caspase-8 and -10 have a death effector domain …

Various apoptotic pathways exist that can be distinguished by the adapters and initiator caspases involved.

Most apoptotic programs fall into either the extrinsic or intrinsic category.

2.2 EXTRINSIC[4]

We first begin with the extrinsic signalling pathway. As Denny notes;

The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions.

These involve ***death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily.*** *Members of the TNF receptor family share similar cyteine-rich extracellular domains and have a cytoplasmic domain of about 80 amino acids called the "death domain".*

This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways.

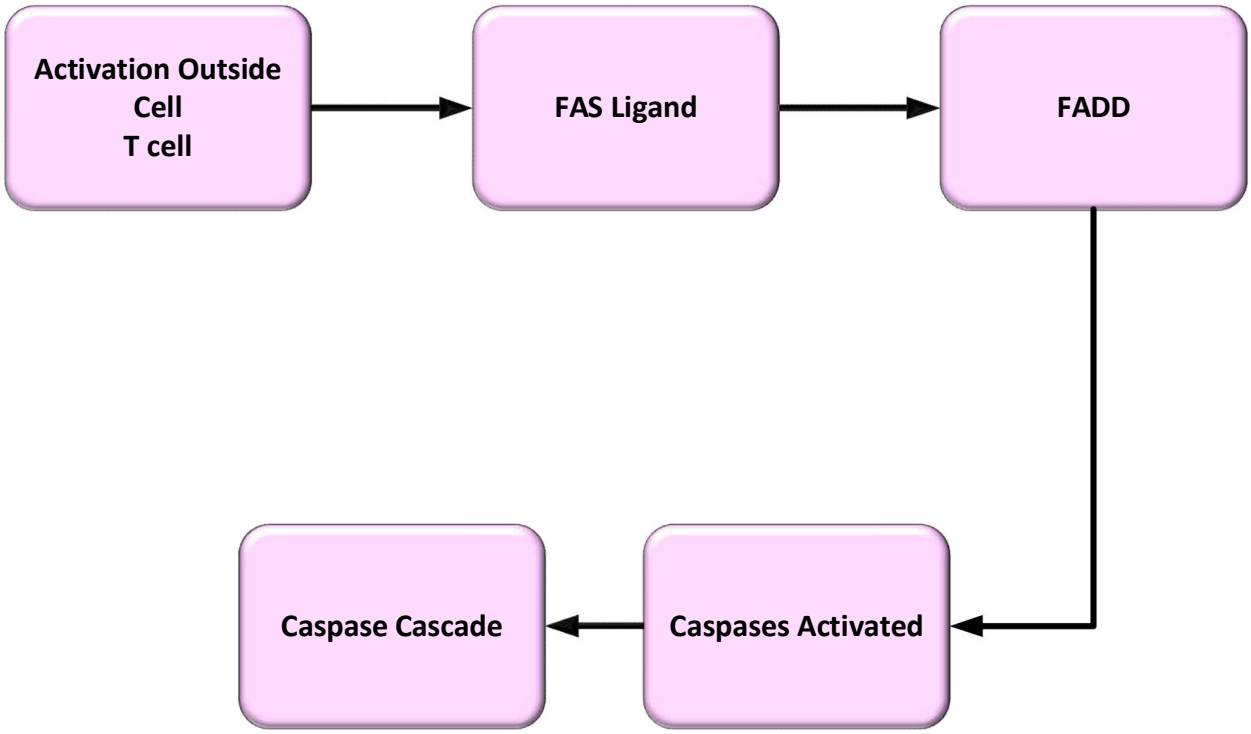
The death ligands and receptors when activated kick off the activation os caspases. They play a critical role.

To date, the best-characterized ligands and corresponding death receptors include FasL/FasR, TNF-α/TNFR1, Apo3L/DR3, Apo2L/ DR4 and Apo2L/DR5. The sequence of events that define the extrinsic phase of apoptosis are best characterized with the FasL/FasR and TNF-α/TNFR1 models. In these models, there is clustering of receptors and binding with the homologous trimeric ligand.

[4] https://www.youtube.com/watch?v=-vmtK-bAC5E

Upon ligand binding, cytplasmic adapter proteins are recruited which exhibit corresponding death domains that bind with the receptors. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD and the binding of TNF ligand to TNF receptor results in the binding of the adapter protein TRADD with recruitment of FADD and RIP.
FADD then associates with procaspase-8 via dimerization of the death effector domain. At this point, a death-inducing signaling complex (DISC) is formed, resulting in the auto-catalytic activation of procaspase-8

The following graphic depicts the steps involved in the extrinsic process.



As McIlwain et al note:

The Extrinsic Pathway of Apoptosis. Extrinsic apoptosis is triggered by extracellular cues delivered in the form of ligands binding to death receptors (DRs).

Death receptors are members of the tumor necrosis factor (TNF) superfamily and include TNF receptor-1 (TNFR1), CD95 (also called Fas and APO-1), death receptor 3 (DR3), TNF-related apoptosis-inducing ligand receptor-1 (TRAIL-R1; also called DR4), and TRAIL-R2 (also called DR5 in humans). Rodents have only one TRAIL-R protein and it resembles DR5

Death receptor ligands include TNF, CD95-ligand (CD95-L; also called Fas-L), TRAIL (also called Apo2-L), and TNF-like ligand 1A (TL1A).

The binding of a DR ligand to a DR causes the monomeric procaspase-8 protein to be recruited via its DED to the death inducing signaling complex (DISC) formed at the cytoplasmic tail of the engaged DR that also includes the adapter protein FAS-associated death domain (FADD) or TNFR-associated death domain (TRADD).

Recruitment of caspase-8 monomers results in dimerization and activation. Cells from gene-targeted mice deficient for caspase-8 (casp82/2 mice) are thus resistant to DR-induced apoptosis, as are cells from mutant mice lacking either FADD or TRADD, which are specifically defective for TNF-a-mediated apoptosis .

The outcome of DR-mediated activation of caspase-8 depends on the cell type. In so-called type I cells, caspase-8 initiates apoptosis directly by cleaving and thereby activating executioner caspases. In type II cells, caspase-8 must first activate the intrinsic apoptotic pathway (discussed below) to induce efficient cell death. Type I and II cells differ in their content of intracellular inhibitor of apoptosis proteins (IAPs), which block executioner caspase function unless suppressed by proteins released from the mitochondria

2.3 INTRINSIC

The second implementation is the intrinsic signalling pathway. As Denny notes:

The intrinsic signaling pathways that initiate apoptosis involve a diverse array of non-receptor-mediated stimuli that produce intracellular signals that act directly on targets within the cell and are mitochondrial-initiated events.

The stimuli that initiate the intrinsic pathway produce intracellular signals that may act in either a positive or negative fashion. Negative signals involve the absence of certain growth factors, hormones and cytokines that can lead to failure of suppression of death programs, thereby triggering apoptosis. In other words, there is the withdrawal of factors, loss of apoptotic suppression, and subsequent activation of apoptosis.

Other stimuli that act in a positive fashion include, but are not limited to, radiation, toxins, hypoxia, hyperthermia, viral infections, and free radicals.

All of these stimuli cause changes in the inner mitochondrial membrane that results in an opening of the mitochondrial permeability transition (MPT) pore, loss of the mitochondrial transmembrane potential and release of two main groups of normally sequestered pro-apoptotic proteins from the intermembrane space into the cytosol (Saelens et al., 2004). The first group consists of cytochrome c, Smac/DIABLO, and the serine protease HtrA2/Omi.

These proteins activate the caspasedependent mitochondrial pathway. Cytochrome c binds and activates Apaf-1 as well as procaspase-9, forming an "apoptosome"). The clustering of procaspase-9 in this manner leads to caspase-9 activation. Smac/DIABLO and HtrA2/Omi are reported to promote apoptosis by inhibiting IAP (inhibitors of apoptosis proteins) activity. Additional mitochondrial proteins have also been identified that interact with and suppress the action of IAP however gene knockout experiments suggest that binding to IAP alone may not be enough evidence to label a mitochondrial protein as "pro-apoptotic".

The second group of pro-apoptotic proteins, AIF, endonuclease G and CAD, are released from the mitochondria during apoptosis, but this is a late event that occurs after the cell has committed to die. AIF translocates to the nucleus and causes DNA fragmentation into ~50– 300 kb pieces and condensation of peripheral nuclear chromatin. This early form of nuclear condensation is referred to as "stage I" condensation.

Endonuclease G also translocates to the nucleus where it cleaves nuclear chromatin to produce oligonucleosomal DNA fragments (Li et al., 2001). AIF and endonuclease G both function in a caspase-independent manner. CAD is subsequently released from the mitochondria and translocates to the nucleus where, after cleavage by caspase-3, it leads to oligonucleosomal DNA fragmentation and a more pronounced and advanced chromatin condensation. This later and more pronounced chromatin condensation is referred to as "stage II" condensation.

The control and regulation of these apoptotic mitochondrial events occurs through members of the Bcl-2 family of proteins. The tumor suppressor protein p53 has a critical role in regulation of the Bcl-2 family of proteins, however the exact mechanisms have not yet been completely elucidated.

The Bcl-2 family of proteins governs mitochondrial membrane permeability and can be either pro-apoptotic or antiapoptotic. To date, a total of 25 genes have been identified in the Bcl-2 family. Some of the anti-apoptotic proteins include Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, BAG, and some of the pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk. These proteins have special significance since they can determine if the cell commits to apoptosis or aborts the process. It is thought that the main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome c release from the mitochondria via alteration of mitochondrial membrane permeability.

As Vitale et al note:

Intrinsic apoptosis is a type of regulated cell death (RCD) initiated by perturbations of the extracellular or intracellular microenvironment including (but not limited to) DNA damage, endoplasmic reticulum or oxidative stress, growth factor withdrawal, and microtubular alterations.

The critical step is mitochondrial outer membrane permeabilization (MOMP).

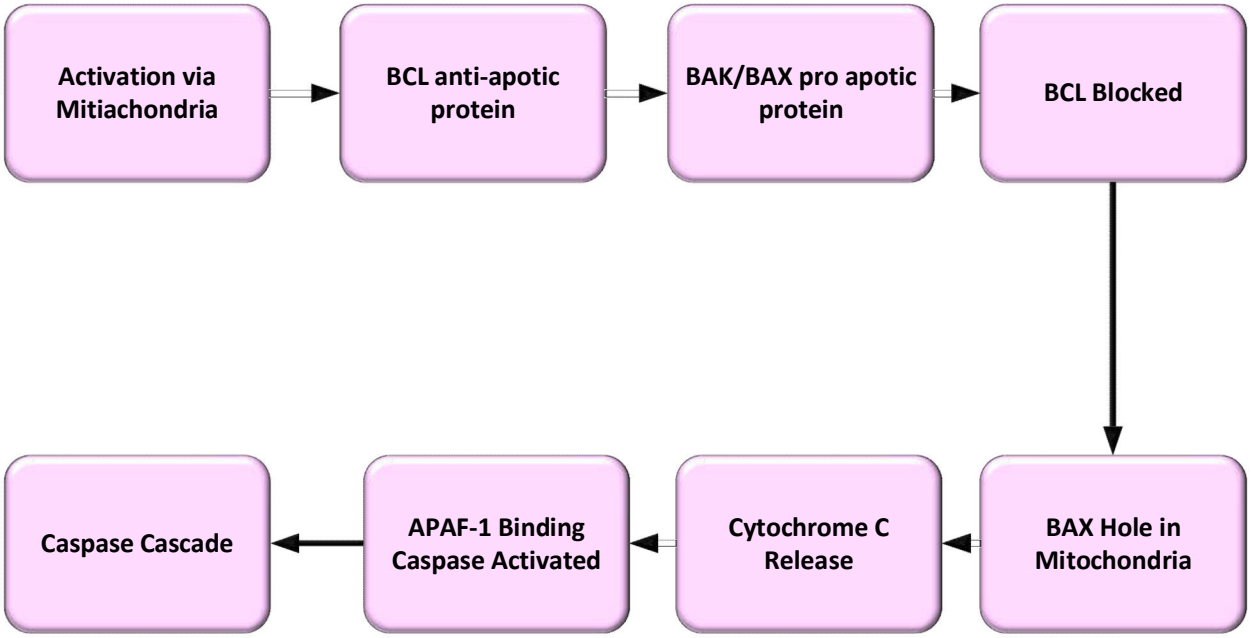
MOMP is modulated by the activity of multiple pro-apoptotic and anti-apoptotic members of the BCL2, apoptosis regulator (BCL2) protein family.

In response to apoptotic stimuli, MOMP leads to the sequential activation of the initiator caspase 9 (CASP9) and then executioner caspases CASP3 and CASP7. Two functionally distinct classes of pro-apoptotic BCL2 proteins have been identified. The first class encompasses the apoptotic activators BCL2 associated X, apoptosis regulator (BAX), BCL2 antagonist/killer 1 (BAK1), and BCL2 family apoptosis regulator (BOK). Once activated by apoptotic stimuli, BAX, BAK1 and BOK induce MOMP by generating pores across the outer mitochondrial membrane (OMM).

These pro-apoptotic factors promote the release into the cytosol of several apoptogenic factors, including cytochrome c, somatic (CYCS) and diablo IAP-binding mitochondrial protein (DIABLO; also known as second mitochondrial activator of caspases, SMAC) [1070]. CYCS exerts apoptogenic activity by associating with apoptotic peptidase activating factor 1 (APAF1) and pro-CASP9 to generate a complex known as the apoptosome, leading to sequential activation of CASP9 and executioner caspases CASP3 and CASP7 . DIABLO/SMAC contributes to CASP3 and CASP7 activation by associating with and inhibiting X-linked inhibitor of apoptosis (XIAP) and other members of the inhibitor of apoptosis (IAP) protein family that restrain caspase activation.

The second class of pro-apoptotic BCL2 proteins (known as BH3-only proteins [1073]) include BCL2 associated agonist of cell death (BAD), BCL2 binding component 3 (BBC3; best known as p53-upregulated modulator of apoptosis, PUMA), BCL2 interacting killer (BIK), BCL2 like 11 (BCL2L11; best known as BIM), Bcl2 modifying factor (BMF), BH3 interacting domain death agonist (BID), BCL2 interacting protein harakiri (HRK, also known as DP5), and phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1; best known as NOXA). Of these, caspase-cleaved BID (tBID), BIM, PUMA, and NOXA have been reported to also be able to promote BAX and BAK1 activation through a direct interaction with these proteins at mitochondria

The graphic below depicts the overall implementation of the intrinsic pathway.



Now cIlwain et al note:

Intrinsic apoptosis is also known as mitochondrial apoptosis because it depends on factors released from the mitochondria.

This pathway is activated by a vast array of cellular stresses, including growth factor deprivation, cytoskeletal disruption, DNA damage, accumulation of unfolded proteins, hypoxia, and many others.

It can also be activated by developmental signals that instruct cells to die, such as hormones. The initiator caspase responsible for the intrinsic apoptosis pathway is caspase-9, which is activated by dimerization induced when the caspase-9 CARD domain binds to the adapter protein apoptotic protease-activating factor-1 (APAF1). Both APAF1 and caspase-9 exist in a resting cell as cytosolic, inactive monomers.

A cell experiencing stress first releases cytochrome c from the mitochondria. The binding of cytochrome c to the WD domain of the APAF1 monomer leads to a conformational change that exposes a nucleotide-binding site in the nucleotidebinding and oligomerization (NACHT) domain of APAF1. The nucleotide deoxy-ATP (dATP) binds to this site and induces a second conformational change in APAF1 that exposes both its oligomerization and CARD domains. Seven such activated APAF1 monomers then assemble into an oligomeric complex, the center of which contains the CARDs that recruit and activate caspase-9.

The complex containing cytochrome c, APAF1, and caspase-9 has been termed the apoptosome. Cytochrome c has a long established role in electron transport, and it was shown in 2000 that mammalian cells lacking cytochrome c could not activate caspases in response to mitochondrial pathway stimulation.

However, it was not until … formally established that the electron transport function of cytochrome c is independent of its ability to engage APAF1 and induce apoptosome formation and caspase activation. Cells from a knockin mouse mutant in which cytochrome c was mutated at lysine 72, a key residue for APAF1 interaction, were able to carry out electron transport but not apoptosis.

The critical role of intrinsic apoptosis in mammalian development is illustrated by the phenotypes of gene-targeted mice deficient for components of this pathway.

During the development of the normal brain, apoptosis is critical for culling massive amounts of brain cells to allow selection of those making the best neural connections …

2.4 EXECUTION PATHWAY

A third approach is the execution pathway. A step following one of the previous discussed. Following Denny we have:

The extrinsic and intrinsic pathways both end at the point of the execution phase, considered the final pathway of apoptosis.

It is the activation of the execution caspases that begins this phase of apoptosis. Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins.

Caspase-3, caspase-6, and caspase-7 function as effector or "executioner" caspases, cleaving various substrates including cytokeratins, PARP, the plasma membrane cytoskeletal protein alpha fodrin, the nuclear protein NuMA and others, that ultimately cause the morphological and biochemical changes seen in apoptotic cells.

Caspase-3 is considered to be the most important of the executioner caspases and is activated by any of the initiator caspases (caspase-8, caspase-9, or caspase-10). Caspase-3 specifically activates the endonuclease CAD. In proliferating cells CAD is complexed with its inhibitor, ICAD.

In apoptotic cells, activated caspase-3 cleaves ICAD to release CAD. CAD then degrades chromosomal DNA within the nuclei and causes chromatin condensation.

Caspase-3 also induces cytoskeletal reorganization and disintegration of the cell into apoptotic bodies. Gelsolin, an actin binding protein, has been identified as one of the key substrates of activated caspase-3.

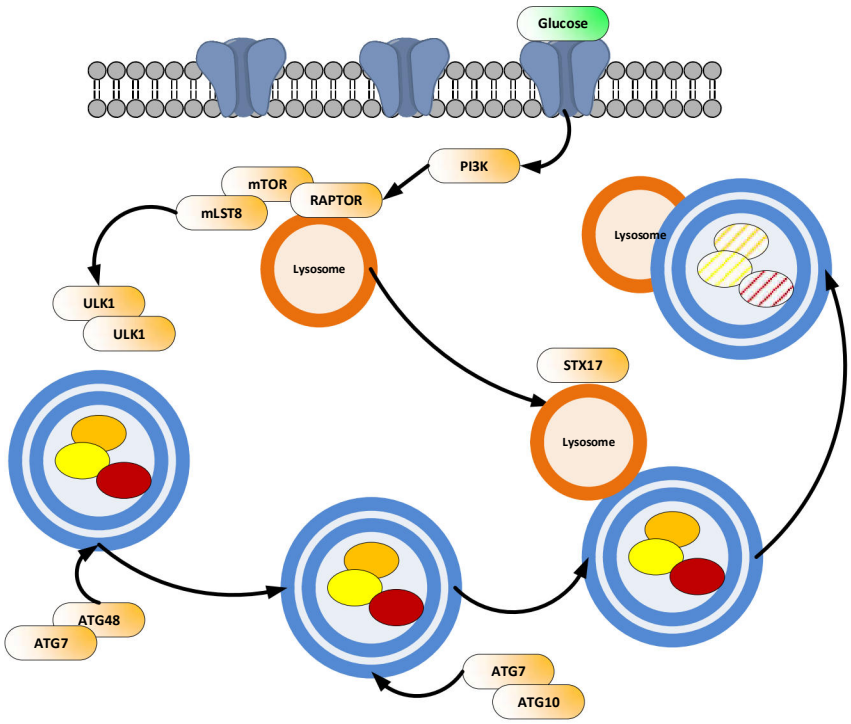
Gelsolin will typically act as a nucleus for actin polymerization and will also bind phosphatidylinositol biphosphate, linking actin organization and signal transduction. Caspase-3 will cleave gelsolin and the cleaved fragments of gelsolin, in turn, cleave actin filaments in a calcium independent manner.

This results in disruption of the cytoskeleton, intracellular transport, cell division, and signal transduction

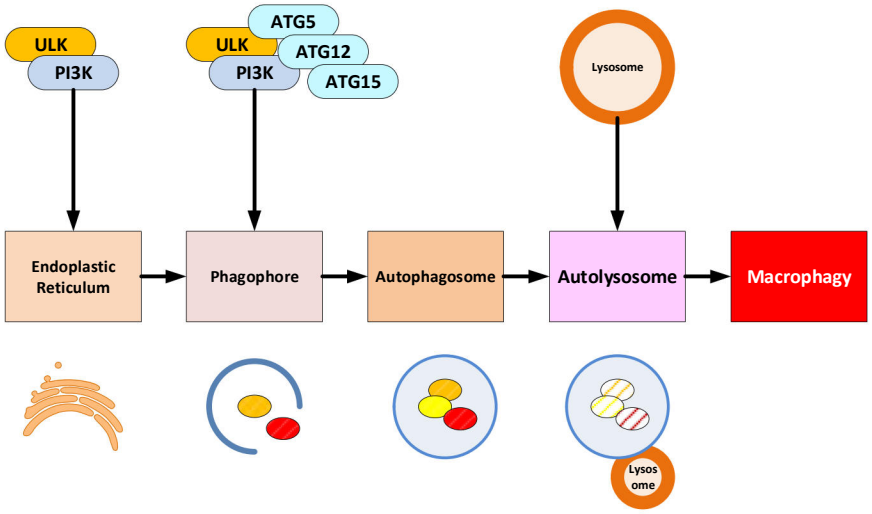
The net result is cell death.

3 AUTOPHAGY AS A PROCESS

Autophagy is dramatically different than apoptosis. Autophagy is a self-immolation process. In many cases it is cell eating itself up due to stress externally or internally. We first commence in understanding autophagy as a process. Unfortunately our understanding starts after selection of a molecule, possibly a protein, is to be lysed. The process of autophagy can be shown in a simple manner as shown below:

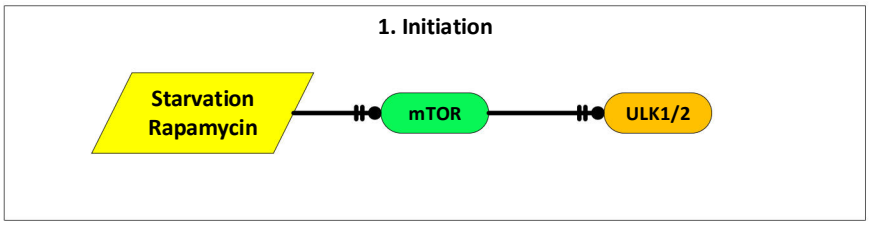


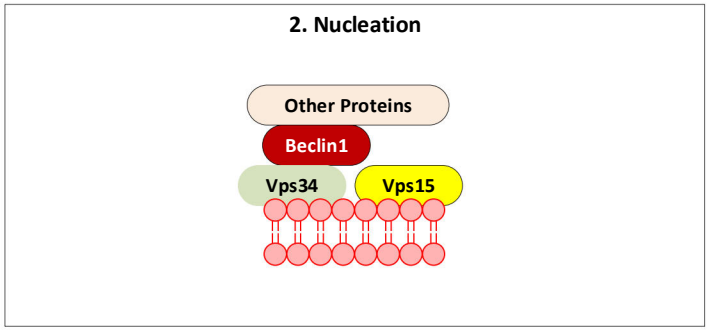
Again we really have no understanding or why and how the selection process was made. Then as a flow with related gene controllers we see as shown below:



Namely it starts in the ER and then by driving genes, PI3K and the ATG complexes we move it into a phagophore and when combined with a lysosome we get degradation and replenishment.

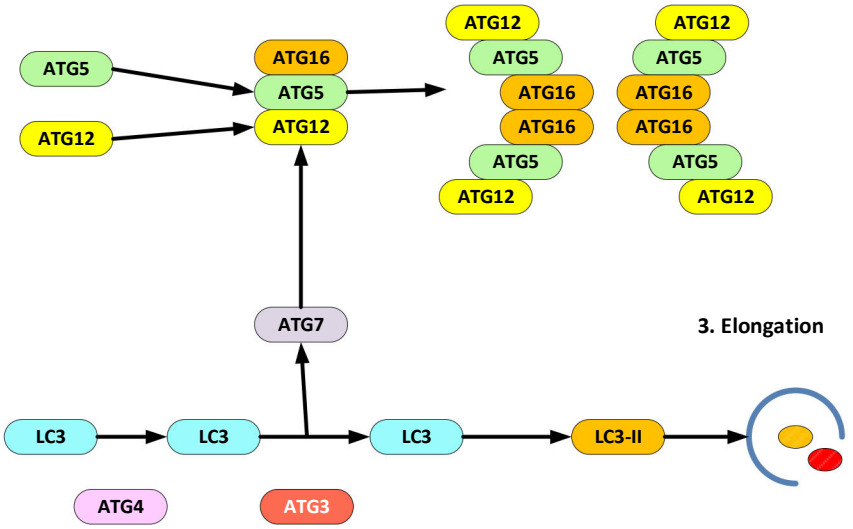
The following figures are based upon Kang et al as modified[5] The first two steps are depicted below. First is some form of initiation. But, and this is critical, the initiation is not based on the molecule to be lysed but on some exogeneous factor. Here it is starvation. Once initiated, the process of building up a cell wall commences as shown below.:



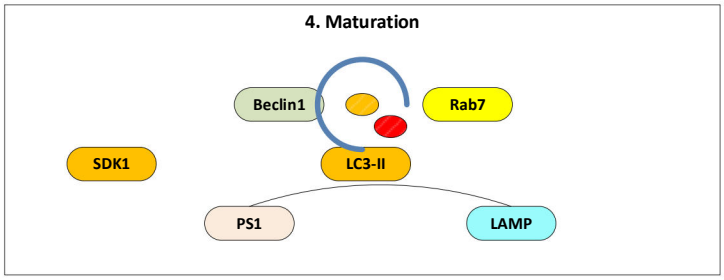


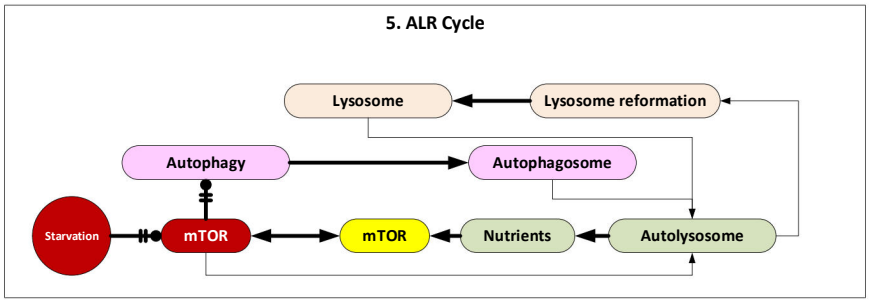
Now after the beginning of nucleation we need to complete the cell wall for closure. The process involved in that is depicted below. We note a multiple set of ATG genes which we will discuss latter.

[5] Kang et al note: *Stages of autophagy. (a) Different types of autophagy. LC3-II is a marker of Atg5/Atg7-dependent autophagy, whereas Rab-9 is a marker of Atg5/Atg7-independent autophagy. (b) The initiation is sustained by activation of ULK1 and ULK2 complexes, which are inhibited by mTOR. (c) The nucleation depends on Beclin 1-Vps34-Vps15 core complexes and other proteins. (d) The elongation of the phagophore is mediated by two ubiquitin-like conjugation systems that together promote the assembly of the ATG16L complex and the processing of LC3. PE, phosphatidylethanolamine. (e) The maturation is promoted by LC3, Beclin 1, the lysosomal membrane proteins LAMP-1 and LAMP-2, the GTP-binding protein RAB7, the ATPase SKD1, the cell skeleton, the pH of lysosomes and possibly presenilin 1 (PS1). (f) Autophagic lysosome reformation (ALR) cycle. mTOR signaling is inhibited during initiation of autophagy, but reactivated by prolonged starvation. Reactivation of mTOR is autophagy-dependent and requires the degradation of autolysosomal products. Increased mTOR activity attenuates autophagy and generates proto-lysosomal tubules and vesicles that extrude from autolysosomes and ultimately mature into functional lysosomes, thereby restoring the full complement of lysosomes in the cell .*



Finally we can see the details of the final step below along with a simplified overview of the process..





Now as Rao notes:

There are three pathways induced by autophagy-related genes and associated enzymes.

The first of these, macroautophagy, is used primarily to eradicate cell waste such as damaged organelles and unused protein.

This involves the generation of a double membrane around the waste to form the autophagosome, which subsequently moves through the cytoplasm and fuses with the lysosome. Once fused, the contents of the autophagosome is degraded the acidic lysosome hydrolases of the lysosome.

The second process, microautophagy, is involved in the direct engulfment of cytoplasmic material into the lysosome, transpiring through inward folding of the lysosomal membrane or cellular protrusion.

The third process, chaperone mediated autophagy, involves the recognition and binding of the Hsc70-containing substrate to the Hsc70-chaperone protein to form the chaperone-substrate or chaperone complex.

As a result, this complex binds to the lysosomal membrane-bound protein, which acts as a chaperone-mediated autophagy receptor allowing the complex cell entry. The unfolded substrate proteins translocate across the lysosomal membrane via lysosomal hsc70 chaperone assistance. Autophagy has diversified functionality.

For example, it was observed in yeast cells, that nutrient deprivation induced significant autophagy. This assists in redundant protein degradation, where resultant amino acids are recycled for cell survival protein synthesis. To date, approximately fifteen APG genes have been identified in the autophagy of yeast cells. Murine studies revealed that APG7 has been implicated in nutrient suppressed autophagy. Moreover, autophagy was impaired in APG7 deficient mice when exposed to nutrient suppressed environment. Xenophagy is the autophagic mortification process of organisms causing infections, an earnest aspect of distinctive immunity.

Intracellular pathogens like Mycobacterium tuberculosis are targeted for the degradation by the same cellular and regulatory mechanisms, targeting host mitochondrial for degradation, eventuating in the destruction of the invasive organism.

Furthermore, stimulation of autophagy in infected cells could help overcome this phenomenon, reinstating pathogen degradation. Autophagy degrades damaged organelles, cell membranes as well as proteins and recycles the degraded components. A failure in this process is believed to be one of the main reasons for the accumulation of cell damage and aging. Cancer is propelled when several metabolic pathways that regulate cell differentiations are obstructed.

Autophagy plays a role in both tumor suppression and tumor survival.

Hence autophagy inhibition leads to cell survival instead of cell death. The protein, Beclin1, regulates autophagy, and is essential for phosphatidylinositol 3-phosphate production, effecting multifarious lysosomal and endosomal functionalities. Overexpression of Beclin 1 reduces tumor development.

In necrosis, cell death leads to chronic inflammation. In autophagy, inflammation is evaded as the cellular components are not released into the surrounds, thereby prevents inflammation and fosters in the protection against tumor cell formation. Thus autophagy plays a key role in tumor suppression. In cancer cells, cell death is avoided through the inhibition of autophagy related genes. The metabolic energy is elevated by the offset of autophagy functionality. Moreover, autophagy inhibition has been shown to elevate the effectiveness of anticancer therapy.

4 AUTOPHAGY AND CANCER

What is the role of autophagy in cancer. It is complex. More than a decade ago it was noted (see Marx):

Suspicions that autophagy plays a role in cancer first arose about 3 decades ago when researchers noted that cancer cells seemed deficient in the process compared to normal cells. They made this determination either by measuring the rates of degradation of long-lived proteins or by looking for the characteristic double-membraned vacuoles that form in cells undergoing autophagy. These vacuoles encircle the cellular cargo destined for degradation and then fuse with lysosomes, which carry a host of enzymes for digesting proteins and other materials. …

The answer to the question of whether inducers of autophagy will be good or bad for cancer therapies may vary depending on the nature of the cancer, the drug, or both. The drug temozolomide (TMZ), which is currently in clinical trials for treating gliomas, provides an illustration of this kind of complexity. Kondo's team found that a drug that inhibits the late stages of autophagy enhanced TMZ's antitumor effects, whereas a different drug that blocks an early stage of autophagy suppressed them. "We have to understand all the players to predict whether a therapy [promoting autophagy] will protect the cells or kill them," Kimchi says. Obviously, autophagy researchers still have their work cut out for them.

According to Marinkovic et al:

Autophagy as a Tumor-Suppressor Mechanism. As mentioned above, autophagy plays a complex dual role in tumorigenesis and is consequently the reason why development of the autophagy-based cancer therapy is so demanding.

The preliminary molecular mechanisms of tumor initiation and progression are much more complex than the mechanism of the actual disease development.

The basis for any malignant cell transformation is the activation of a protooncogene or the inactivation of tumor suppressor genes. However, a large number of studies confirm that cancer cells also have altered core autophagy regulators, where either their expression levels or genetic information is altered. Knowing this, autophagy could behave similarly as a tumor suppressor (acting to prevent tumor initiation) or as a tumor promoter to ensure tumor longevity via apoptosis inhibition….

Autophagy as a Tumor-Promoter Mechanism.

While autophagy has a tumor-suppressing role in the early stage of carcinogenesis, in advanced cancers, it often acts as a tumor survival or even tumor promoter mechanism. This is mostly due to the fact that tumor cells are resistant to extremely stressful conditions, that is, nutrient and oxygen deprivation, within the tumor tissue.

These conditions are even more rigorous in the central part of the solid tumors where the autophagy level is significantly higher than on the periphery. This suggests that autophagy in

some tumors also acts as an adaptive mechanism enabling their advancement in the absence of the key survival factors. Yang et al. found that an increased autophagy level in mouse pancreatic cancer led to tumor regression and prolonged lifespan.

Another support of the theory comes from studies where the knockouts of core autophagy proteins, ATG5, ATG7, or FIP200, were analyzed. Wei et al. analyzed and reported that with the removal of FIP200 in human breast cancer mouse models, tumor initiation and progression was suppressed.

The analysis of multiple cancers showed the overexpression of ATG5 in gastric and prostate cancers while overexpression of ATG7 was seen in bladder cancer. In contrast, mice with systemic mosaic deletion of Atg5 or Atg7 developed benign liver adenomas that do not progress to adenocarcinoma or metastasize. Taken together, these results demonstrate the involvement of core autophagy proteins in tumor development and progression. In conclusion, depending on the type of tumor and its developmental stage, activation or inactivation of autophagy can contribute differently to tumorigenesis.

Reduced autophagy can contribute to tumor progression, whereas increased autophagy may be a mechanism for tumor survival under hypoxic, metabolic, or therapeutic stress conditions. Thus, the modulation of the autophagy process is a promising, but complex, therapeutic strategy for the enhancement of anticancer treatments.

A better understanding of the autophagy in tumor models is crucial in identifying new and effective therapeutic strategies for cancer treatment.

Next, we summarize the preclinical and clinical usage of autophagy modulators in common cancer types. Here, we outline a brief overview of current knowledge on modifications of the core autophagy machinery in pancreatic, breast, hepatocellular, colorectal, and lung cancers that represent a promising strategy for the future of drug development. Currently, these tumors represent an example of the successful application of autophagy modulation in preclinical models, which proved to be valuable for novel clinical trials.

The chosen cancer types represent an example where the dual role of autophagy, both tumor promoter and tumor suppressor, has been established. Moreover, depending on the autophagy role in cancer development and progression, specific preclinical tumor models have been designed specifically aimed at activating or to inhibiting autophagy.

Most recent studies on autophagy inhibition have reported on the use of late-stage autophagy inhibitors, CQ or HCQ, which effectively inhibit autophagosomelysosome fusion. However, their usage as autophagy inhibitors in cancer treatment is quite controversial. Current data proposes that the inhibition of late autophagy by CQ or HCQ might not be the only mechanism of their action in cancer. Hence, they can affect tumor cell survivability through the inhibition of immune cell action against tumor cells or influence the permeabilization of the lysosomal membrane thus affecting apoptosis. CQ is often cytotoxic at high doses and can promote cell cycle arrest or DNA damage that induces cancerogenesis.

Now Abraham et al note:

Macroautophagy, often simply (and hereafter) referred to as autophagy, is the best studied autophagic process and focus of this review. Autophagy has been recognized to be a pro-survival mechanism at times of cellular stress including starvation. Besides, increasing evidence indicated the importance of autophagy in in the pathogenesis of several diseases including cancer. But, its role in cancer is more complex and still controversial; it appears to be tumor suppressive during tumorigenesis, but contributes to tumor cell survival during cancer progression.

Besides, autophagic capacity was shown to significantly affect responses of cancer cells to anticancer agents and radiation. Even though there is still a gap about how autophagy is regulated in cancer, it appears to provide a promising target for cancer treatment. This review aimed at examining the multiple roles of autophagy as a novel target for cancer therapy...

There is now increasing evidence that autophagy has complex and paradoxical roles in tumorigenesis, tumor progression and cancer therapeutics. Currently, there is a consensus that tumor suppressive functions of autophagy act during tumor initiation, and as a survival strategy by established tumors to cope with diverse stresses of the microenvironment that are encountered during tumor progression and metastasis ….

Not only do mutations of the autophagy gene promote tumorigenesis, but autophagy is also positively regulated by the tumor suppressor genes and negatively regulated by the oncogenic pathways.

Oncogenes like Akt and Ras inhibit autophagy primarily by activating the mTOR signaling pathway. Conversely, tumor suppressor PTEN which inhibit PI3K/Akt/mTOR-C1 pathway can activate autophagy.

Therefore, mutations in PTEN result in constitutive activation of the pathway, suppression of autophagy, and may contribute to tumor formation. Other tumor suppressors such as TSC1, TSC2, p53, and liver kinase B1 (LKB1) stimulate autophagy through their inhibitory effects on mTOR-C1. To this end, number mechanisms could clarify the tumor suppressive roles of autophagy, including prevention of oxidative stress and genomic instability, inhibition of necrosis and inflammation, promotion of cancer cell death, modulation of antitumor immune response, maintenance of normal stem cells and degradation of oncogenic proteins…

As Hu et al have noted:

Accumulating evidence indicates a context-dependent role of autophagy in cancer. Autophagy may function as a tumor-suppressive mechanism during early tumorigenesis, but its role in advanced cancer remains unclear.

Direct evidence showing the tumor suppressor function of autophagy comes from the fact that certain ATG-proteins, such as Beclin-1, exhibit an anti-oncogenic function. Inactivation of autophagy-related genes, such as Beclin-1, leads to increased tumorigenesis in mice while overexpression of these genes (Beclin-1, Atg5) inhibit the formation of human breast tumors in mouse models.

The tumor suppressor function of Beclin-1 is supported by the genetic evidence that Beclin-1 is monoallelically deleted in breast, ovarian, and prostate tumors. Distinct to the role of autophagy in tumorigenesis, it is widely accepted that autophagy is required for the survival of established cancers.

In this regard, autophagy inhibitors could be useful as cancer therapeutics. However, regression of tumor xenografts derived from a large number of human cancer cell lines is not detected upon inhibition of autophagy. Although the autophagy inhibitor chloroquine (CQ) suppressed growth of cancer cell lines, whether its effect is autophagy-dependent remains elusive. Taken together, whether autophagy should be inhibited or activated remains controversial.

From Hayat:

The discovery that the autophagic-related gene Beclin 1 suppresses tumor growth stimulated significant interest from cancer biologists in this previously unexplored therapeutic process. This interest has resulted in both intensive and extensive research efforts to understand the role of autophagy in cancer initiation, progression, and suppression.

Pharmacological or genetic inactivation of autophagy impairs KRAS-mediated tumorigenesis. It has been shown that transmembrane protein VMP1 (vacuole membrane protein 1), a key mediator of autophagy, is a transcriptional target of KRAS signaling in cancer cells.

It regulates early steps of the autophagic pathway. In fact, KRAS requires VMP1 not only to induce but also to maintain autophagy levels in cancer. PI3K-AKT1 is the signaling pathway mediating the expression and promoter activity of VMP1 upstream of the GLI3-p300 complex.

The Beclin 1 gene is deleted in -40% of prostate cancers, -50% of breast cancers, and -75% of ovarian cancers. In addition, reduced expression of Beclin 1 has been found in other types of cancers, including human colon cancer, brain tumors, hepatocellular carcinoma, and cervical cancer. It can be concluded that a defective autophagic process is clearly linked to cancer development.

However, it should be noted that the role of autophagy in cancer development is exceedingly complex. In tumorigenesis, autophagy is a double-edged sword acting as either a tumor suppressor or a supporter of cancer cell survival, depending on the stimulus and cell type.

Thus, autophagy can function as an anticancer or pro-cancer mechanism.

In the latter case, autophagy enables tumor cells to survive stressors in the tumor microenvironment. Indeed, some types of cancer cells induce autophagy as a means of adapting to the unfavorable tumor microenvironment, which is characterized by hypoxia, limited nutrients, and metabolic stress. Autophagy, in addition, may block the toxicity of certain anticancer drugs.

Autophagy is associated with resistance to chemotherapeutics such as 5-fluorouracil and cisplatin. It is recognized that tumors and the immune systems are intertwined in a

competition where tilting the critical balance between tumor-specific immunity and tolerance can finally determine the fate of the host.

It is also recognized that defensive and suppressive immunological responses to cancer are exquisitely sensitive to metabolic features of rapidly growing tumors.

On the other hand, autophagy may increase the effectiveness of anticancer radiotherapy. It is known that some malignancies become relatively resistant to repeated radiotherapy, and may eventually recover self-proliferative capacity. This problem can be diminished by inducting autophagy through Beclin 1 overexpression in conjunction with radiotherapy. It is known that autophagy enhances the radio-sensitization of cancer cells rather than protecting them from radiation injury and cell death.

It is also known that autophagy inhibits the growth of angiogenesis in cancer cells. It should also be noted that autophagic cell death occurs in many cancer types in response to various anticancer drugs. In other words, autophagy can serve as a pathway for cellular death. Based on the two opposing roles of autophagy, it is poised at the intersection of life and death. It is apparent that we need to understand and modulate the autophagy pathway to maximize the full potential of cancer therapies.

5 MTOR

mTOR plays a significant role in the process of autophagy. We examine its functions and then move to its role in autophagy. The flow shown below demonstrates the position of mTOR. As NCBI notes[6]:

The protein encoded by this gene belongs to a family of phosphatidylinositol kinase-related kinases. These kinases mediate cellular responses to stresses such as DNA damage and nutrient deprivation. This protein acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex[7].

NCBI (via KEGG) notes[8]:

Autophagy (or macroautophagy) is a cellular catabolic pathway involving in protein degradation, organelle turnover, and non-selective breakdown of cytoplasmic components, which is evolutionarily conserved among eukaryotes and exquisitely regulated. This progress initiates with production of the autophagosome, a double-membrane intracellular structure of reticular origin that engulfs cytoplasmic contents and ultimately fuses with lysosomes for cargo degradation. Autophagy is regulated in response to extra- or intracellular stress and signals such as starvation, growth factor deprivation and ER stress. Constitutive level of autophagy plays an important role in cellular homeostasis and maintains quality control of essential cellular components.

The pathways below demonstrate this discussion. mTOR is facilitated by Raptor, Deptor, PRAS40 and mLSTS. This is the core of the mTOR signalling pathway. The ATG genes then facilitate the autophagy process when activated via this mTOR complex. mTOR plays a significant role in cell survival and proliferation.

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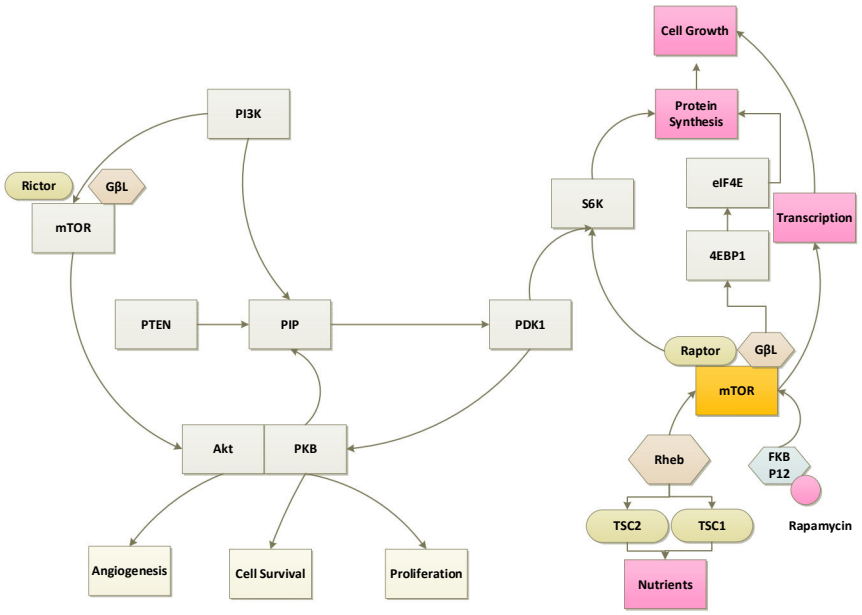
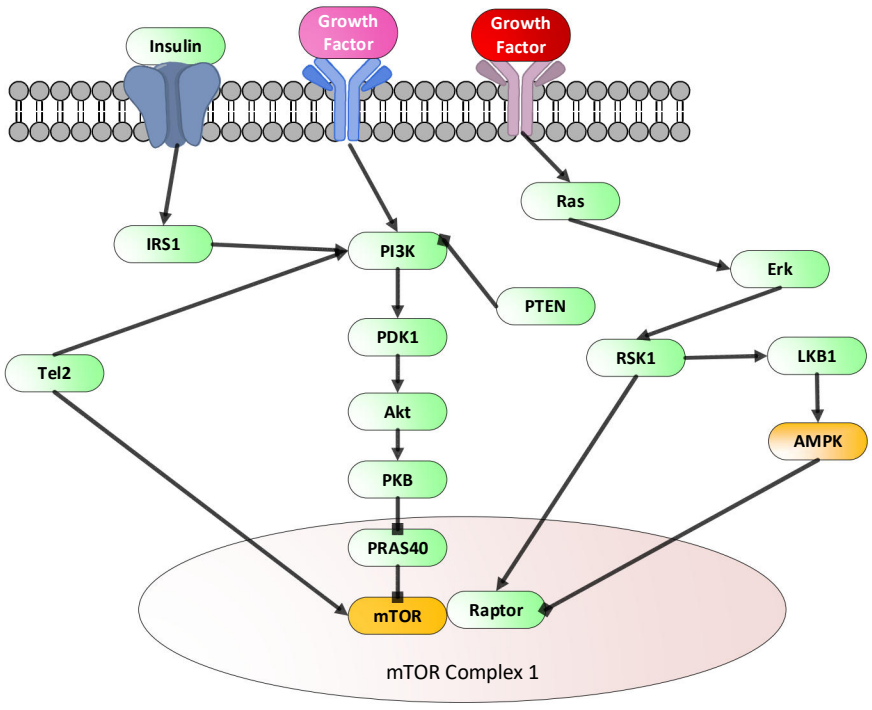
[6] https://www.ncbi.nlm.nih.gov/gene/2475

[7] From NCBI, *The mammalian target of rapamycin (mTOR) also known as mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) is a protein which in humans is encoded by the FRAP1 gene. mTOR is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription. mTOR belongs to the phosphatidylinositol 3-kinase-related kinase protein family.*

[8] https://www.ncbi.nlm.nih.gov/biosystems/83058?Sel=geneid:2475#show=genes also see the KEGG pathway https://www.kegg.jp/pathway/hsa04140



Currently the pathways which control many cancers are also pathways in which mTOR plays a role. In the following figure we depicts some of these which have obtained significant clinical interest and attention.



From Paquette et al:

The mechanistic target of rapamycin (mTOR) is a serine-threonine protein kinase that can be divided into two functionally and biochemically distinct complexes, mTORC1 and mTORC2. Both are implicated in growth factor sensing, but mTORC1 is generally the one associated with

cell proliferation and cancer progression when deregulated. Significant progress was made in recent years to understand mTORC1 response to growth factors, such as insulin and insulin-like growth factor. ....

Macroautophagy (referred to as autophagy hereafter), the cellular self-degradation process, plays an important role in energy supply, particularly during development and in response to nutrient stress. It is a process through which cargo is delivered to double-membrane vesicles, termed autophagosomes, which fuse with the lytic compartment and release the inner vesicle into the lumen, leading to the degradation of cell components and the recycling of cellular building blocks.

This intracellular mechanism is conserved in eukaryotes from yeast to complex multicellular organisms, and its dysfunction has been implicated in many human diseases, including myopathy, neurodegeneration, and cancer, as well as resistance to pathogen infection.

At the molecular level, autophagy plays a context dependent pro-survival or pro-death role by regulating different signaling pathways, including ***p53, Bax-interacting factor-1 (Bif-1), Beclin 1 (BECN1), ultraviolet irradiation resistance-associated gene (UVRAG), mTOR, protein kinase B (Akt), B-cell lymphoma 2 (Bcl-2), Ras, and Class I PI3K (PI3KI) in cancer****. The focus of this part of the review will be mainly on mTOR pathways; however, these pathways are interconnected and they can integrate into an autophagy-related cancer network that could ultimately affect the fate of cancer cells.*

Among several components involved in the tight regulation of autophagy, mTORC1, but not mTORC2, has been shown to be a key player in coordinating the respective anabolic and catabolic processes in response to environmental and physiological stresses. Studies have shown that mTORC1 inhibition increases autophagy, whereas stimulation of mTORC1 reduces this process. mTORC2 was reported to indirectly suppress autophagy through the activation of mTORC1.

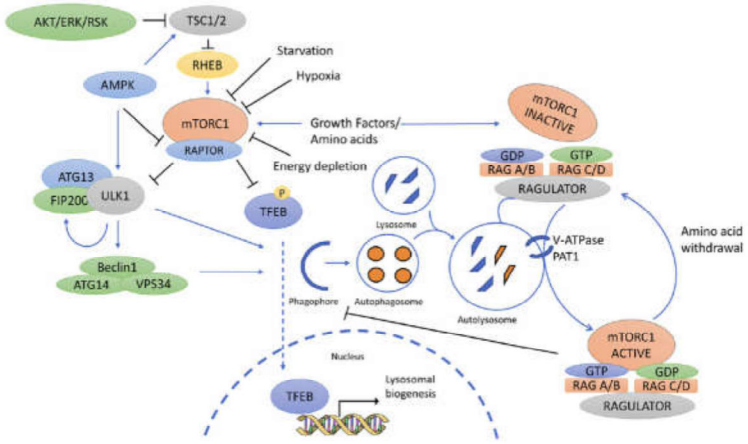
The PI3K signaling axis activates mTORC2, which, in turn, phosphorylates AKT at two different sites, leading to AKT/mTORC1 signaling axis activation. Further studies are required to determine whether there is a direct role for mTORC2 in autophagy regulation. In mammals, and under nutrient-rich conditions, it was reported by three independent groups that mTORC1 controls autophagy through the regulation of a protein complex composed of unc-51-like kinase 1 (ULK1), autophagy-related gene 13 (ATG13), and focal adhesion kinase family-interacting protein of 200 kDa (FIP200) through directly phosphorylating and suppressing this kinase complex required to initiate autophagy.

mTORC1 was reported to directly phosphorylate and suppress this kinase complex required to initiate autophagy.

Conversely, nutrient withdrawal stimulates the ULK1/ATG13/FIP200 complex formation and initiates autophagy via ULK1 auto-phosphorylation and phosphorylation of its binding partners. In line with these findings, rapamycin-induced inhibition of mTORC1 was shown to enhance the kinase activity of ULK1, while mTORC1 activation through Rheb overexpression potently represses ULK1. Subsequent studies further identified Ser758 in the human protein as the major

mTORC1-mediated inhibitory phosphorylation site on ULK1, leading to the complex dissociation and autophagy repression.

In addition to phosphorylation of ULK1, mTORC1 was also shown to indirectly inhibit autophagy through the phosphorylation of autophagy/Beclin-1 regulator 1 (AMBRA1), preventing ubiquitination of ULK1 by TNF receptor-associated factor 6, an E3 ubiquitin protein ligase (TRAF6), which, under starvation conditions, causes ULK1 self-association, stabilization, and enhancement of its kinase activity…



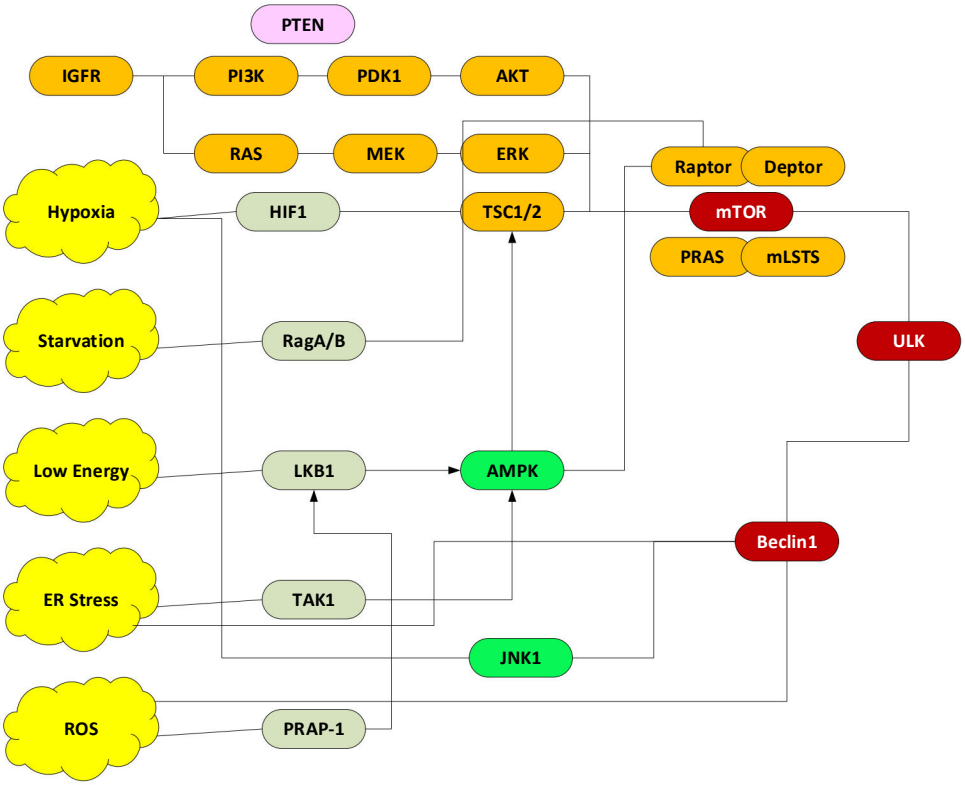
6 BECLIN 1 OR ATG6

Beclin1 is a key gene regulating autophagy and we have seen it referenced extensively already. It also is known as ATG6 although the human form is Beclin1. We details some of its main functions herein.

As NCBI notes[9]:

This gene encodes a protein that regulates autophagy, a catabolic process of degradation induced by starvation. The encoded protein is a component of the phosphatidylinositol-3-kinase (PI3K) complex which mediates vesicle-trafficking processes. This protein is thought to play a role in multiple cellular processes, including tumorigenesis, neurodegeneration and apoptosis.

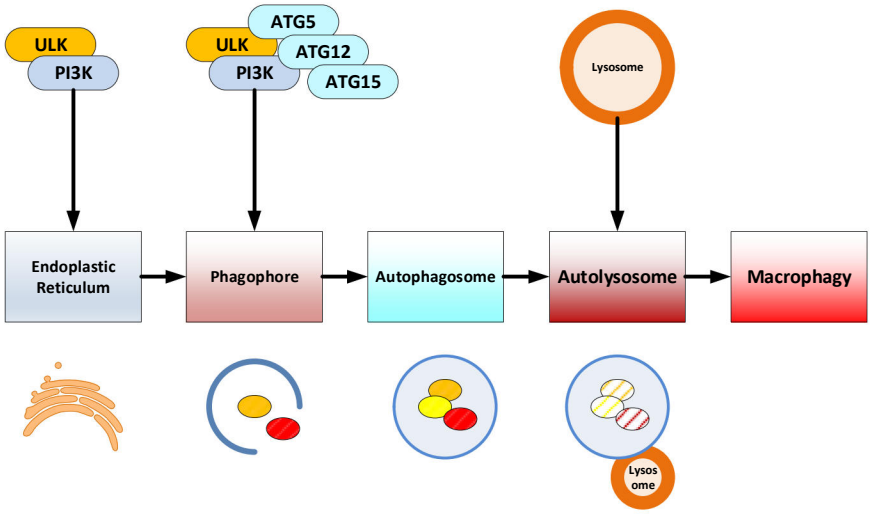
The detailed pathway interaction is shown in KEGG[10] and we show an abbreviated form below. It should then be connected to a previous figure we have included in the following. This figure depicts the multiple drivers for generating autophagy.



Then as noted the following processes ensue. Note that mTOR and Beclin1 drive ULK1/2[11] which in turn drives the processes below. ULK1 is an autophagy activating kinase:

[9] https://www.ncbi.nlm.nih.gov/gene/8678

[10] https://www.kegg.jp/pathway/hsa04140
[11] https://www.ncbi.nlm.nih.gov/gene/8408



As Kang et al note:

Beclin 1, the mammalian orthologue of yeast Atg6, has a central role in autophagy, a process of programmed cell survival, which is increased during periods of cell stress and extinguished during the cell cycle. It interacts with several cofactors (Atg14L, UVRAG, Bif-1, Rubicon, Ambra1, HMGB1, nPIST, VMP1, SLAM, IP3R, PINK and survivin) to regulate the lipid kinase Vps-34 protein and promote formation of Beclin 1-Vps34-Vps15 core complexes, thereby inducing autophagy. In contrast, the BH3 domain of Beclin 1 is bound to, and inhibited by Bcl-2 or Bcl-XL. This interaction can be disrupted by phosphorylation of Bcl-2 and Beclin 1, or ubiquitination of Beclin 1. Interestingly, caspase-mediated cleavage of Beclin 1 promotes crosstalk between apoptosis and autophagy.

Beclin 1 dysfunction has been implicated in many disorders, including cancer and neurodegeneration. Here, we summarize new findings regarding the organization and function of the Beclin 1 network in cellular homeostasis, focusing on the cross-regulation between apoptosis and autophagy.

7 ATG GENES

There is a collection of ATG genes which act to facilitate autophagy. We have discussed some of them as found in humans and herein we discuss several others. From Ohsumi we have noted regarding the ATG genes:

A set of genes encoding machinery essential for the unique membrane dynamics of autophagosome formation
Why are so many genes are yet to be unidentified?
Most researchers interested in "essential genes" in extremely rich medium, such as YEPD.
ATG mutants can grow normally and show little phenotype under growing conditions.

What is encoded by the ATG genes?
Cloning of ATG genes
Sequencing of ATG genes
Identification of Atg proteins → A group of novel uncharacterized genes, no hint about protein function.
18 Atg proteins required for autophagosome formation

Thus there are a collection of some 18 ATG genes involved in this process. We shall examine a few and detail them later.

7.1 ATG1, ATG13, ATG11, ATG20, ATG17 AND ATG24

As Meiling-Wesse notes:

The induction and regulation of autophagy is a largely unknown process. Many components and its exact mechanism have yet to be discovered. What is known is that it involves the protein Tor (Target of Rapamycin). Tor is a phosphatidylinositol kinase related serine/threonine protein kinase. It is involved in nutrient sensing and cell growth and coordinates a number of aspects of cell physiology.

The nutrient sensing function of Tor regulates autophagy. When Tor is inhibited either by nutrient depletion or treatment with rapamycin autophagy is induced. The kinase activity of Tor hyper phosphorylates directly or indirectly the protein Atg13. Atg13 is part of the Atg1 complex. Atg1 and Atg13 are phosphoproteins that are needed for regulating between autophagy and the Cvt pathway. When Tor is active Atg13 is hyperphosphorylated.

This hyperphosphorylation prevents Atg13 from undergoing an interaction with Atg1. Though when Tor is inhibited, Atg13 is partially dephosphorylated. Hypophosphorylated Atg13 then has a higher affinity to Atg1, which activates autophagy and the formation of larger transport vesicles, autophagosomes. The Atg1 complex involves components that are specific to either the autophagy or the Cvt 1 Figure pathway; only Atg1 is involved in both. Therefore Atg1 is considered the activating switch between both pathways…

Note here we show the role of ATG1, ATG13, ATG11, ATG20, ATG17 and ATG24. This is a total of six of the ATG products.

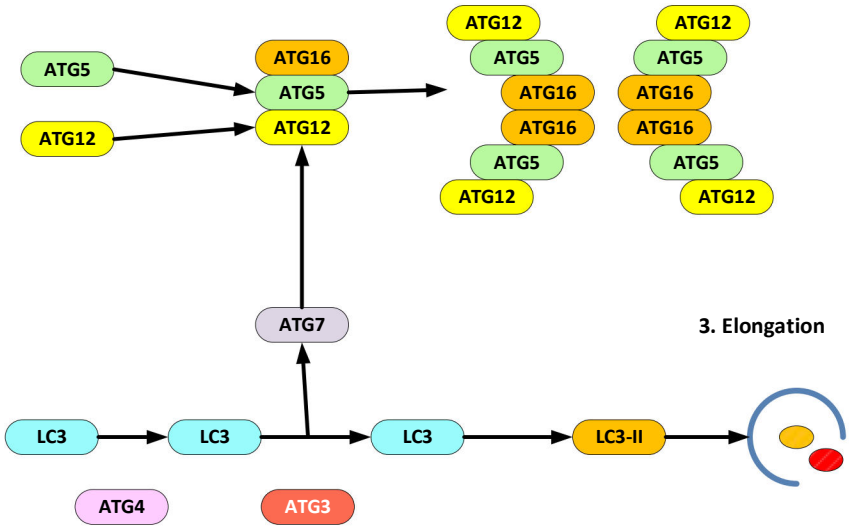
7.2 AGT12, ATG3, ATG8, ATG10, ATG5 AND ATG16

We continue from Meiling-Wesse:

It has been shown that a 350 kDa complex, comprising of a multimer of Atg12, Atg5 and Atg16, is needed for the formation of autophagosomes and Cvt-vesicles. The conjugation of Atg12 to Atg5 proceeds in an ubiquitin like process ...

First cytosolic Atg12 is activated by Atg7, its E1 activator. Second the activated Atg12 is covalently conjugated to Atg5 by its E2 conjugating protein Atg10. Atg5 is found to localized to the PAS in wild type cells as observed using the Atg5-YFP fluorescent protein. Another protein ***Atg16*** *multimerizes through a coiled coil domain to itself and then rapidly links over* ***Atg5*** *to the* ***Atg12-Atg5*** *conjugate. This forms a tetramer of* ***Atg12-Atg5·Atg16****.*

This multimeric complex is created before the induction of autophagy during nutrient rich conditions. It is not known how this complex is needed for the formation of transport vesicles but it might function as a transient coat, since proteins from this complex are not found to mark completed autophagosomes or Cvt vesicles. This complex has also been shown to be necessary for the lipidation of Atg8, the second ubiquitin like conjugating cascade.



7.3 AGT8, ATG4

Again from Meiling-Wesse:

Atg8 was discovered as a low copy suppressor of Atg4 (39) and was found to be essential for the maturation of Ape1 not only during autophagy but also during the Cvt pathway. Atg8 was also identified as the first starvation induced protein of autophagy and a vesicle labelling protein. The cytosolic Atg8, processed by Atg4,… The protease Atg4 cleaves the C-terminal arginine

residue revealing a glycine residue. This modification is necessary for the membrane association of Atg8. In GFP-Atg8 studies, Atg8 is found to be located as a cytosolic pool and at a point on the vacuolar membrane, the PAS (34). After starvation induction Atg8 was found within the vacuolar lumen (42),(34). In the absence of Atg4, Atg8 does not localized to the PAS. As in the case of Atg12, Atg7 is also the E1 activator of Atg8 (43).

The then modified Atg8 is covalently conjugated to phosphatidylethanol-amine (PE) by Atg3, its conjugating E2 enzyme. This step is dependent on the 350 kDa complex, although it is not known how. Lipidated Atg8, Atg8-PE, is then integrated into the double lipid bilayer of the forming autophagosome or Cvt vesicle. After the transport vesicle is completed, Atg8 is found attached inside and outside of the membrane.

Atg4 then cleaves Atg8 from the outer surface allowing it to be recycled, while the Atg8 attached to the inner membrane is trapped within the vesicle and is transported to the vacuole where it is degraded with the cargo. This transfer of Atg8 to the vacuole can be used for observing how autophagosomes travel to the vacuole. Using GFP-Atg8, the transport, degradation and release of free-GFP can be utilized in determining the ability and rate of autophagy in mutant strains.

7.4 ATG SUMMARY

The following is a summary of ATG for humans. Some references are complete others need significant research.

Gene	Function	Reference
ATG1		https://www.ncbi.nlm.nih.gov/gene/3641175
ATG12	Autophagy is a process of bulk protein degradation in which cytoplasmic components, including organelles, are enclosed in double-membrane structures called autophagosomes and delivered to lysosomes or vacuoles for degradation. ATG12 is the human homolog of a yeast protein involved in autophagy	https://www.ncbi.nlm.nih.gov/gene/9140
ATG13	he protein encoded by this gene is an autophagy factor and a target of the TOR kinase signaling pathway. The encoded protein is essential for autophagosome formation and mitophagy.	https://www.ncbi.nlm.nih.gov/gene/9776
ATG16	The protein encoded by this gene is part of a large protein complex that is necessary for autophagy, the major process by which intracellular components are targeted to lysosomes for degradation. Defects in this gene are a cause of susceptibility to inflammatory bowel disease type 10 (IBD10). Several transcript variants encoding different isoforms have been found for this gene	https://www.ncbi.nlm.nih.gov/gene/55054
ATG17	The protein encoded by this gene interacts with signaling pathways to coordinately regulate cell growth, cell proliferation, apoptosis, autophagy, and cell migration. This tumor suppressor also enhances retinoblastoma 1 gene expression in cancer cells. Alternative splicing results in multiple transcript variants encoding distinct isoforms	https://www.ncbi.nlm.nih.gov/gene/9821
ATG20		https://www.ncbi.nlm.nih.gov/gene/851445
ATG2B	This gene encodes a protein required for autophagy. The encoded protein is involved in autophagosome formation. A germline duplication of a region that includes this gene is associated with predisposition to myeloid malignancies.	https://www.ncbi.nlm.nih.gov/gene/55102
ATG3	This gene encodes a ubiquitin-like-conjugating enzyme and is a component of ubiquitination-like systems involved in	https://www.ncbi.nlm.nih.gov/gene/64422

	autophagy, the process of degradation, turnover and recycling of cytoplasmic constituents in eukaryotic cells. This protein is known to play a role in regulation of autophagy during cell death. A pseudogene of this gene is located on chromosome 20. Alternative splicing results in multiple transcript variants encoding different isoforms.	
ATG4B	Autophagy is the process by which endogenous proteins and damaged organelles are destroyed intracellularly. Autophagy is postulated to be essential for cell homeostasis and cell remodeling during differentiation, metamorphosis, non-apoptotic cell death, and aging. Reduced levels of autophagy have been described in some malignant tumors, and a role for autophagy in controlling the unregulated cell growth linked to cancer has been proposed. This gene encodes a member of the autophagin protein family. The encoded protein is also designated as a member of the C-54 family of cysteine proteases. Alternate transcriptional splice variants, encoding different isoforms, have been characterized.	https://www.ncbi.nlm.nih.gov/gene/23192
ATG5	The protein encoded by this gene, in combination with autophagy protein 12, functions as an E1-like activating enzyme in a ubiquitin-like conjugating system. The encoded protein is involved in several cellular processes, including autophagic vesicle formation, mitochondrial quality control after oxidative damage, negative regulation of the innate antiviral immune response, lymphocyte development and proliferation, MHC II antigen presentation, adipocyte differentiation, and apoptosis.	https://www.ncbi.nlm.nih.gov/gene/9474
ATG6 BECN1 Beclin 1	This gene encodes a protein that regulates autophagy, a catabolic process of degradation induced by starvation. The encoded protein is a component of the phosphatidylinositol-3-kinase (PI3K) complex which mediates vesicle-trafficking processes. This protein is thought to play a role in multiple cellular processes, including tumorigenesis, neurodegeneration and apoptosis. Alternative splicing results in multiple transcript variants	https://www.ncbi.nlm.nih.gov/gene/8678
ATG7	This gene encodes an E1-like activating enzyme that is essential for autophagy and cytoplasmic to vacuole transport. The encoded protein is also thought to modulate p53-dependent cell cycle pathways during prolonged metabolic stress. It has been associated with multiple functions, including axon membrane trafficking, axonal homeostasis, mitophagy, adipose differentiation, and hematopoietic stem cell maintenance. Alternative splicing results in multiple transcript variants	https://www.ncbi.nlm.nih.gov/gene/10533
ATG8	Broad expression in brain	https://www.ncbi.nlm.nih.gov/gene/23710
ATG9		https://www.ncbi.nlm.nih.gov/gene/3635239
ATH10	Autophagy is a process for the bulk degradation of cytosolic compartments by lysosomes. ATG10 is an E2-like enzyme involved in 2 ubiquitin-like modifications essential for autophagosome formation: ATG12 (MIM 609608)-ATG5 (MIM 604261) conjugation and modification of a soluble form of MAP-LC3 (MAP1LC3A; MIM 601242), a homolog of yeast Apg8, to a membrane-bound form	https://www.ncbi.nlm.nih.gov/gene/83734

8 SOME OTHER GENES

It is worth examining a few other related genes. First we examine SIRT1 based upon NCBI. From NCBI we have for SIRT1[12]:

SIRT1: This gene encodes a member of the sirtuin family of proteins, homologs to the yeast Sir2 protein. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class I of the sirtuin family. Alternative splicing results in multiple transcript variants.

The regulatory nature of SIRT1 is a key element in its functioning in PCa. We will examine how this may function shortly.

And relating to SOD2[13]:

SOD2 superoxide dismutase 2, mitochondrial: This gene is a member of the iron/manganese superoxide dismutase family. It encodes a mitochondrial protein that forms a homotetramer and binds one manganese ion per subunit. This protein binds to the superoxide byproducts of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen.

Mutations in this gene have been associated with idiopathic cardiomyopathy (IDC), premature aging, sporadic motor neuron disease, and cancer. Alternate transcriptional splice variants, encoding different isoforms, have been characterized.

And for PARK2 we have[14]:

The precise function of this gene is unknown; however, the encoded protein is a component of a multiprotein E3 ubiquitin ligase complex that mediates the targeting of substrate proteins for proteasomal degradation. Mutations in this gene are known to cause Parkinson disease and autosomal recessive juvenile Parkinson disease. Alternative splicing of this gene produces multiple transcript variants encoding distinct isoforms. Additional splice variants of this gene have been described but currently lack transcript support.

From Powell et al we have as more detailed discussion of the functions of Sirt1:

The Sirtuin family of proteins (SIRT) encode a group of evolutionarily conserved, NAD-dependent histone deacetylases, involved in many biological pathways. SIRT1, the human

[12] http://www.ncbi.nlm.nih.gov/gene/23411

[13] http://www.ncbi.nlm.nih.gov/gene/6648

[14] http://www.ncbi.nlm.nih.gov/gene/5071

homologue of the yeast Silent Information Regulator 2 (Sir2) gene, de-acetylates histones, p300, p53, and the androgen receptor. Autophagy is required for the degradation of damaged organelles and long-lived proteins, as well as for the development of glands such as the breast and prostate. Herein, homozygous deletion of the Sirt1 gene in mice resulted in prostatic intraepithelial neoplasia (PIN) associated with reduced autophagy.

Genome-wide gene expression analysis of Sirt1/ prostates demonstrated that endogenous Sirt1 repressed androgen responsive gene expression and induced autophagy in the prostate. Sirt1 induction of autophagy occurred at the level of autophagosome maturation and completion in cultured prostate cancer cells. These studies provide novel evidence for a checkpoint function of Sirt1 in the development of PIN and further highlight a role for SIRT1 as a tumor suppressor in the prostate.

The autophagy cleans up the cells and brings them back to a normal stasis. The recognition of Powell et al regarding the role of Sirt1 is key. They continue:

The role of SIRT1 in regulating prostate gland formation and androgen signaling in vivo was previously unknown. SIRT1is expressed in several cell types in the prostate gland including basal cells, luminal cells, and stromal cells. Given the evidence that SIRT1 functions as a tissue-specific regulator of cellular growth and that SIRT1 inhibits tumor cell line growth in nude mice, we sought to determine the role of endogenousSirt1 in regulating prostate gland development. Genome-wide expression profiling of Sirt1/ mice prostates and their littermate controls identified a molecular, genetic signature regulated by endogenous Sirt1.

The above clearly shows the understanding of the function of Sirt1. Note that the Powell work was in 2010 so that this understanding has been available for a while.

This signature highlights the ability of Sirt1 to inhibit androgen signaling and apoptosis in the prostate, while promoting autophagy. The Sirt1/ prostates demonstrated epithelial hyperplasia and PIN suggesting that Sirt1 promotes autophagy and inhibits prostate epithelial cell proliferation in vivo.

The above demonstrates the ability of Sirt1 to control androgen signalling. This also is a key factor in controlling prostate health.

Gene expression analysis further demonstrated that loss of endogenous Sirt1 inhibited autophagy. At a higher level of resolution, our studies demonstrated that SIRT1 antagonized DHT-mediated inhibition of autophagy in the prostate. Autophagy allows for degradation of proteins and organelles and is induced by nutrient withdrawal, rapamycin (inhibition of mTOR signaling), and hormone signaling.

Our findings are consistent with prior studies demonstrating that SIRT1 induces autophagy by deacetylating ATG5, ATG7, and ATG8 and inhibits AR signaling via deacetylation of the AR. Comparisons with previously published studies identified an overlap of 12.45% between genes regulated by endogenous Sirt1 and those targeted by androgens in the prostate gland and in prostate cancer cells. These results are consistent with prior findings that Sirt1 inhibits ligand-dependent AR signaling and gene expression in vitro

Again we come back to the role of autophagy. Perhaps the buildup of protein segments may act as normal cell blockage, inhibiting normal expression and control. The autophagy allows for a return to such normality. The emphasize this issue as follows:

The role of autophagy in cancer was proposed over 20 years ago. Autophagy appears to be essential for tumor suppression as well as for cell survival. Autophagy plays a prosurvival function for cancer cells during nutrient deprivation or when apoptotic pathways are compromised, a phenotype often accompanied by inflammation.

Again we see the putative role of inflammation. This appears to be a significant factor in PCa and the suppression of genes which deal with the remnants of inflammation seem to be a key benchmark in PCa progression. They continue:

In contrast, upon disruption of tumor suppressors, autophagy adopts a pro-death role with apoptotic pathways. In prostate, breast, ovarian, and lung cancer, loss of Beclin1 or inhibition of Beclin1 by the BCL-2 family of proteins causes defective autophagy, increased DNA damage, metabolic stress, and genomic instability.

These cancers also display neoplastic changes and increased cell proliferation, unlike cells overexpressing Beclin1, which undergo apoptosis. Loss of PTEN, p53, ATG4, ATG5, and MAP1LC31 (ATG8) are linked to tumorigenesis, whereas upregulation of PI3K, AKT, BCL-2, and mTOR are associated with inhibition of autophagy and the promotion of tumorigenesis.

Prostate cancer onset and progression are correlated strongly with aging and SIRT1 function governs aging in multiple species. Further studies will be required to determine whether this checkpoint function of Sirt1 in regard to prostate growth is linked to its role in organismal aging.

From Shackelford et al we have additional insights including pathway control issues as follows:

AMPK has recently been shown to increase sirtuin 1 (SIRT1) activity by increasing cellular NAD+ levels, resulting in the regulation of many downstream SIRT1 targets, including FOXO3 and peroxisome proliferator activated receptor-γ co-activator 1 (PgC1; also known as PPARgC1A), both of which have also been proposed to be direct substrates of AMPK46,76. As SIRT1 is also implicated in tumorigenesis, this connection between AMPK and SIRT1 might further explain how nutrients control cell growth. AMPK also suppresses mTOR-dependent transcriptional regulators to inhibit cell growth and tumorigenesis.

Two mTORC1-regulated transcription factors involved in cell growth are the sterol-regulatory element-binding protein 1 (SReBP1) and hypoxiainducible factor 1a (HIF1α). SReBP1 is a sterolsensing transcription factor that drives lipogenesis in many mammalian cell types. mTORC1 signalling is required for nuclear accumulation of SReBP1 and the induction of SReBP1 target genes78, and this can be inhibited by rapamycin or AMPK agonists

From Hines et al we have an expression of Sirt1 in terms of overall cell control:

The NAD + -dependent deacetylase SIRT1 is an evolutionarily conserved metabolic sensor of the Sirtuin family that mediates homeostatic responses to certain physiological stresses such as nutrient restriction. Previous reports have implicated fluctuations in intracellular NAD + concentrations as the principal regulator of SIRT1 activity. However, here we have identified a cAMP-induced phosphorylation of a highly conserved serine (S434) located in the SIRT1 catalytic domain that rapidly enhanced intrinsic deacetylase activity independently of changes in NAD + levels.

Attenuation of SIRT1 expression or the use of a nonphosphorylatable SIRT1 mutant pre- vented cAMP-mediated stimulation of fatty acid oxidation and gene expression linked to this path- way. Overexpression of SIRT1 in mice significantly potentiated the increases in fatty acid oxidation and energy expenditure caused by either pharmacological b -adrenergic agonism or cold exposure. These studies support a mechanism of Sirtuin enzymatic control through the cAMP/PKA pathway with important implications for stress responses and maintenance of energy homeostasis

From Dominy et al we have:

From an evolutionary perspective, the nutrient-dependent control of protein acetylation through acetyltransferases and deacetylases is highly conserved and is a major mechanism for coupling metabolic activity with carbon/energy availability. The regulated acetylation of PGC-1a by GCN5 and Sirt1 is an excellent example: PGC-1a acetylation by GCN5 is favored under conditions of nutrient/energy abundance, whereas deacetylation by Sirt1 is favored under conditions of nutrient dearth and high energy demand

Finally Brooks and Gu state:

SIRT1 is a multifaceted, NAD+-dependent protein deacetylase that is involved in a wide variety of cellular processes from cancer to ageing. The function of SIRT1 in cancer is complex: SIRT1 has been shown to have oncogenic properties by down regulating p53 activity, but recent studies indicate that SIRT1 acts as a tumour suppressor in a mutated p53 background, raising intriguing questions regarding its mechanism of action.

Here we discuss the current understanding of how SIRT1 functions in light of recent discoveries and propose that the net outcome of the seemingly opposite oncogenic and tumour-suppressive effects of SIRT1 depends on the status of p53.

They clearly indicate the tumor suppressor role of Sirt1. p53 status is important but the observation above is truly intriguing if it is sustained.

9 OBESITY AND AUTOPHAGY

As we have noted repeatedly, obesity, and obesity accompanied with Type 2 Diabetes is a significant driver of a multiplicity of malignancies. Besides the generation of massive amounts of ROS, the excess uncontrolled glucose and excitation of the IGFR is a major driver for various malignancies.

As Namkoong et al have noted:

ATG1 is one of the first genes isolated to mediate the autophagy response in yeast. Unc-51-like kinase 1 (ULK1) and 2 (ULK2), ATG1's mammalian homologs, are essential for initiation of autophagy. Activity of ULK1 is controlled by two nutrient-regulated protein kinases, AMP-activated kinase (AMPK) and mTOR complex 1 (mTORC1). AMPK is activated upon cellular energy (ATP) depletion while mTORC1 is activated upon nutrient abundance. AMPK is known to inhibit mTORC1 through several distinct mechanisms .

AMPK and mTORC1 produce diametrically opposing effects on ULK1; ULK1 is activated by AMPK mediated phosphorylation but inhibited by mTORC1- mediated phosphorylation. Through these mechanisms, autophagy can be activated by starvation while suppressed during nutritional affluence. Indeed, hepatic autophagy is strongly upregulated during starvation and coordinates liver metabolism to meet the metabolic needs of the organism during the nutritional stringency.

Autophagy was thought to be inactive during obesity because hypernutrition can inhibit AMPK and subsequently activate mTORC1. Indeed, mTORC1 activity is chronically upregulated during obesity, which is associated with increased anabolic metabolism in liver.

Consistent with this premise, initial studies on obese mice showed dramatic downregulation of autophagic activities, associated with reduced expression of ATG5 and ATG7 and the subsequent inhibition in autophagosome biogenesis. Insulin resistance and hyperinsulinemia were also suggested to contribute to autophagy inhibition during obesity. In addition, recent studies suggest that lipotoxic insults can downregulate AMPK signaling, thereby decreasing autophagosome production in macrophages and liver cells. In contrast, other studies involving both human and mouse tissues demonstrated that autophagosomes can accumulate in response to obesity and lipotoxicity in multiple tissues, including liver and adipose tissues.

These findings suggest that the relationship between obesity and autophagy is not as simple as originally speculated. For instance, ER stress, which can be provoked by obesity and lipotoxicity as reviewed above, can induce autophagy through multiple mechanisms.

Obesity per se is an established strong inducer of ER stress in liver and obesity-associated ER stress aggravates fat accumulation, insulin resistance and liver damage. Therefore, it is plausible that, as a defensive mechanism against ER stress-induced damages, cells upregulate autophagy. In fibroblasts, lipotoxic activation of protein kinase C (PKC) can upregulate autophagic flux thereby protecting cells from apoptotic cell death. Other stresses associated with obesity, such as inflammation and oxidative stress, can also upregulate autophagy through various mechanisms. Autophagy induction in this context can be viewed as part of a cellular

defense mechanism that is coordinated to maintain cellular homeostasis under obesity associated stresses.

10 OBSERVATIONS

We can now make some observations regarding autophagy and cancer.

10.1 AUTOPHAGY AS A PROCESS IS SOMEWHAT WELL UNDERSTOOD ONCE IT COMMENCES AND FOLLOWING THROUGH COMPLETION. HOWEVER AUTOPHAGY AS A MEANS TO INHIBIT OR PROMOTE CANCERS DOES NOT SEEM TO BE WELL UNDERSTOOD AT THE INITIATION STAGE.

We have examined several putative autophagic related cancer treatments which we will comment on latter. However most of these are on off approaches and a general systematic approach does not seem forthcoming.

10.2 AUTOPHAGY AS A THERAPEUTIC TARGET MAY HAVE POTENTIAL FOR SILENCING GENE PRODUCTS WHICH FACILITATE THE EXPANSION OF CERTAIN MALIGNANCIES.

For example Baquero et al note:

In chronic myeloid leukemia (CML), ***tyrosine kinase inhibitor (TKI) treatment induces autophagy that promotes survival and TKI-resistance in leukemic stem cells (LSCs).***

In clinical studies hydroxychloroquine (HCQ), the only clinically approved autophagy inhibitor, does not consistently inhibit autophagy in cancer patients, so more potent autophagy inhibitors are needed. We generated a murine model of CML in which autophagic flux can be measured in bone marrow-located LSCs.

In parallel, we use cell division tracing, phenotyping of primary CML cells, and a robust xenotransplantation model of human CML, to investigate the effect of Lys05, a highly potent lysosomotropic agent, and PIK-III, a selective inhibitor of VPS34, on the survival and function of LSCs. We demonstrate that long-term haematopoietic stem cells (LT-HSCs: Lin−Sca-1+c-kit +CD48−CD150+) isolated from leukemic mice have higher basal autophagy levels compared with non-leukemic LT-HSCs and more mature leukemic cells.

Additionally, we present that while HCQ is ineffective, Lys05-mediated autophagy inhibition reduces LSCs quiescence and drives myeloid cell expansion. Furthermore, Lys05 and PIK-III reduced the number of primary CML LSCs and target xenografted LSCs when used in combination with TKI treatment, ***providing a strong rationale for clinical use of second generation autophagy inhibitors as a novel treatment for CML patients with LSC persistence.***

Cristofani et al note regarding prostate cancer:

Within tumour mass, ***autophagy may promote cell survival by enhancing cancer cells tolerability to different cell stresses, like hypoxia, starvation or those triggered by chemotherapic agents****. Because of its connection with the apoptotic pathways,* ***autophagy has been differentially implicated, either as prodeath or prosurvival factor, in the appearance of***

more aggressive tumours. *Here, in three PC cells (LNCaP, PC3, and DU145), we tested how* ***different autophagy inducers modulate docetaxel-induced apoptosis.*** *We selected the mTOR-independent disaccharide trehalose and the mTOR-dependent macrolide lactone rapamycin autophagy inducers. In castration-resistant PC (CRPC) PC3 cells, trehalose specifically prevented intrinsic apoptosis in docetaxel-treated cells. Trehalose reduced the release of cytochrome c triggered by docetaxel and the formation of aberrant mitochondria, possibly by enhancing the turnover of damaged mitochondria via autophagy (mitophagy). In fact, trehalose increased LC3 and p62 expression, LC3-II and p62 (p62 bodies) accumulation and the induction of LC3 puncta. In docetaxel-treated cells, trehalose, but not rapamycin, determined a perinuclear mitochondrial aggregation (mito-aggresomes), and mitochondria specifically colocalized with LC3 and p62-positive autophagosomes.*

In PC3 cells, rapamycin retained its ability to activate autophagy without evidences of mitophagy even in presence of docetaxel. Interestingly, these results were replicated in LNCaP cells, whereas trehalose and rapamycin did not modify the response to docetaxel in the ATG5-deficient (autophagy resistant) DU145 cells. Therefore, autophagy is involved to alter the response to chemotherapy in combination therapies and the response may be influenced by the different autophagic pathways utilized and by the type of cancer cells.

10.3 AUTOPHAGY PRODUCTS MAY ALLOW FOR LIQUID BIOPSY TARGETS FOR THE PURPOSE OF ASCERTAINING DIAGNOSTIC OR PROGNOSTIC TARGETS.

We have discussed liquid biopsy approaches.

10.4 CAN THE GENE AND GENE PRODUCTS IN AUTOPHAGY BE USED AS TARGETS TO MITIGATE CERTAIN TYPES OF CANCERS?

Some effort has been tried on this area and a great deal more is required.

10.5 IS THERE SOME APPROACH THAT CAN BE FACILITATED VIA IMMUNOTHERAPY?

10.6 ARE THERE VIRAL VECTORS WHICH CAN BE EMPLOYED TO FACILITATE AUTOPHAGIC CONTROLS?

10.7 WHAT IS THE IMPACT OF OBESITY AND AUTOPHAGY ON CANCER PRESENTATION?

Obesity has been and is a major source of morbidity and mortality. It has further become a topic with some significant social backlash for a physician. Whereas smoking could be called out and managed obesity has become a personal statement protected by those who often have no understanding of its risks.

Noa Zhang et al note:

Obesity poses a severe threat to human health, including the increased prevalence of hypertension, insulin resistance, diabetes mellitus, cancer, inflammation, sleep apnoea and other chronic diseases. Current therapies focus mainly on suppressing caloric intake, but the efficacy of this approach remains poor.

A better understanding of the pathophysiology of obesity will be essential for the management of obesity and its complications. Knowledge gained over the past three decades regarding the aetiological mechanisms underpinning obesity has provided a framework that emphasizes energy imbalance and neurohormonal dysregulation, which are tightly regulated by autophagy.

Accordingly, there is an emerging interest in the role of autophagy, a conserved homeostatic process for cellular quality control through the disposal and recycling of cellular components, in the maintenance of cellular homeostasis and organ function by selectively ridding cells of potentially toxic proteins, lipids and organelles. Indeed, defects in autophagy homeostasis are implicated in metabolic disorders, including obesity, insulin resistance, diabetes mellitus and atherosclerosis.

…the alterations in autophagy that occur in response to nutrient stress, and how these changes alter the course of obesogenesis and obesity-related complications, are discussed. The potential of pharmacological modulation of autophagy for the management of obesity is also addressed.

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