AUTOPHAGY AND PCA: ON THE ONE HAND AND ON THE OTHER HAND

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This is an extension of a previous study of autophagy. It examines a research paper examining autophagy on prostate cancer, PCa, cells.

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1 INTRODUCTION

We have discussed autophagy previously[1]. We have also discussed metabolism of cancer cells[2]. The melding of these has been examined extensively by White at Rutgers whereas the autophagy process has been similarly examined in detail by Sabatini at MIT/Whitehead. There is an extensive amount of research detailing the autophagy process, relating it to metabolic processes, and finally showing its putative linkage to cancers. In this Note we return to this topic and as with many other such Notes try to assemble a systems approach to these factors in cancer environments.

Let us briefly summarize what we know at a high level.

1. Cancer cells are very active cells. They proliferate significantly and as such demand high energy sources to enable the proliferation.

2. The classic metabolic pathways are used by most cells. Namely the Krebs cycle. However under stress and in cancer cells the Warburg approach is seen.

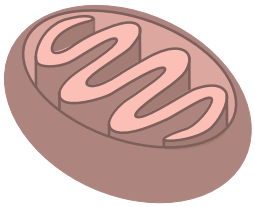
3. Autophagy is generally a normal cell process removing cell waste. However it can be activated to generate energy for proliferation or survival by a multiple set of drivers. Growth factors, insulin, glucose, and other exogeneous factors can activate autophagy. Autophagy is seen in many cancers, and autophagy that drives the rapid proliferation of the cancer cells.

4. The mitochondria are multiple small organelles in the cell which function to produce energy via the Krebs cycle, for example. There can be as many as 2,000 mitochondria in a cell each having its own circular DNA[3]. The DNA may be slightly different.

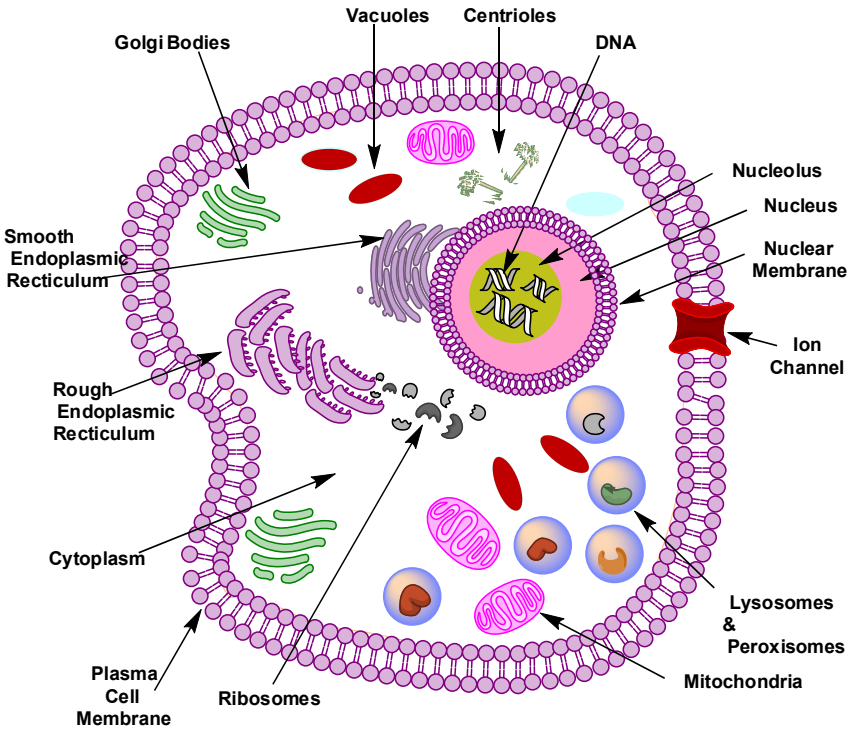
[1] See Autophagy and Cancer, Sept 2018, http://www.telmarc.com/Documents/White%20Papers/154Autophagy.pdf

[2] Glucose, Warburg, Cancer and Pathways, January 2018, http://www.telmarc.com/Documents/White%20Papers/148Warburg.pdf

[3] See Decuypere et al. They provide an interesting discussion of mitochondria and autophagy. Specifically they note: *Autophagy is a conserved delivery pathway to the lysosomes and used for the degradation of long-lived proteins and other macromolecules, protein aggregates, damaged organelles and even foreign pathogens. In stress situations (e.g. nutrient starvation) this process offers the cell a fresh pool of building blocks and has thus a prosurvival function. Although seemingly a cellular process opposite to cell death, prolonged stimulation of autophagy can lead to what is called autophagic cell death. Autophagic cell death should however rather be regarded as cell death with autophagy, instead of cell death by autophagy. In this respect, the autophagic features found in the dying cells are indicative of the cellular attempts to survive during prolonged stress conditions. Cells have to make the decision whether to try to survive (autophagy) or to kill themselves (apoptosis). It is therefore no surprise that important cross-talks exist between these two pathways.*



In fact the multiple mitochondria can make up a substantial portion of the inside of a cell. Graphically we show that below.



Now all of these cellular components participate in the cells homeostasis. But as we can be concerned about DNA replication in the nucleus, we are thousands of times more risky in the mitochondrion. We just make a note of this because every time we have to replicate something mistakes can be troublesome.

5. Cancer cells demonstrate excessive autophagy and disruptions in the normal energy process. Cancer cells have an independence from their original source fabric. They do what they want and go where they wish.

6. Autophagy is a process, it is a complicated process which when activated can provide the cell with energy or other elements required or equally well can destroy and breakdown cell elements. As a process it contains a complex set of internal steps. It also has inputs or drivers and outputs. It sits in the midst of a plethora of cellular pathways.

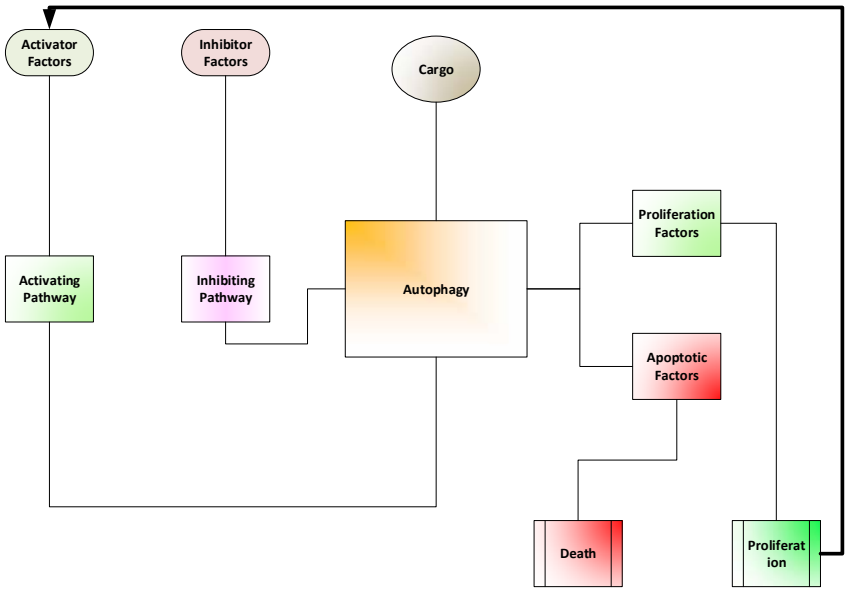
7. Cellular dynamics in a cancer cell are complicated but driven to survive and proliferate. To proliferate they need large sources of energy and that energy comes from autophagy and modified metabolic pathways. Thus there is a nexus between the Warburg effect and autophagy. Moreover they tend to reinforce one another in cancer cells. As Kimmelman and White have noted:

A great deal of evidence implicates the importance of autophagy in the metabolism of various cancers. Recent studies have helped more precisely define the metabolic contributions of autophagy and how these may factor into the pro-tumorigenic activities of this complex process. Interestingly, the involvement of autophagy in metabolism extends beyond the cancer cells themselves, as it appears to play a role in the metabolic crosstalk between tumor and stromal cells in tumor types such as pancreatic cancer. The diverse nature of autophagic cargo allows for the rapid production of needed metabolic fuel sources that can feed into nearly every pathway in central carbon metabolism and beyond.

Given the tremendous stresses that tumor cells undergo, this provides a reservoir to help cope with the ever-changing metabolic needs of a tumor. This includes cell intrinsic stresses, such as the need to maintain energy homeostasis and nucleotide pools during unconstrained proliferation and increased ROS production. On top of these intrinsic stresses, tumor cells encounter metabolic stresses created by harsh conditions in the tumor microenvironment. These include hypoxia, low pH, and decreased nutrient supply due to dysfunctional vasculature. Autophagy provides cells the ability to adapt to a series of stresses, allowing the tumor to continue to proliferate and survive.

We shall examine these issues herein. But again the goal is to attempt to assemble elements of the "system" which accomplishes this task.

In view of developing a systems approach we present a model for a single cell as shown below. We will examine many of the elements contained therein in some detail.



There are the following:

Activating and Inhibiting Factors: These are external factors such as growth factors which attaché to receptors and initiate pathways.

Pathways: These are the Activating and Inhibiting pathways, each of which may have a therapeutic factor.

Autophagy: The process of taking a "cargo" molecule and if activated breaking it down.

Cargo: The target molecule to be broken down

Factors: The results of the breakdown which in turn are used to activate or kill cells.

Feedback: The results in a proliferation mode may be released and in a feedback manner cause multicell proliferation.

2 AN EXAMPLE OF AUTOPHAGY IN CANCERS

The issue we are examining is one where we ask; how does autophagy help and/or hinder the efficacy of chemotherapy on the treatment of prostate cancer (PCa)? As we have noted elsewhere[4], autophagy, the consuming of intracellular elements via lysosome activation and incorporation can initially suppress cancerous cells and then can suppress the chemotherapy that is attempting to inhibit their proliferation and thus act as a facilitator of PCa proliferation.

When examining PCa as a system, namely a set of multiple control points and elements, we need to understand that by changing one setting we may be making the response worse than before, and that autophagy is one element in this overall chain of system elements. From a systems perspective, various elements are part of the overall control. Some enhance and some deter. Understanding these influences then potentially enlighten the path to control and mitigation via therapeutics.

Autophagy is a process. It is in some ways like apoptosis, normal cell death. Except with autophagy there are multiple controls and initiators, and multiple outcomes, some good and some harmful. In trying to understand autophagy we are forced to try to understand the cells not in isolation but as a totality of cells in a living organism. Thus in this note we will try to examine as a benchmark studies with cell lines but at the same time bring to the fore the systemic effects.

In a recent paper by Cristofani et al, which discusses the systems elements in the treatment of PCa. We focus on this paper because of the confluence of three issues; autophagy, chemotherapy, and prostate cancer.

It seems fair to outline the elements of this paper. They are from our perspective as follows:

1. Use three standard in vitro cell lines; LNCaP, PC3 and DU145.

2. Using a glucose based sugar and a therapeutic, docetaxel, ascertain if they each excite autophagy. This is accomplished by examining markers of autophagy.

3. Using rapamycin, do the same testing regarding autophagy.

4. Using docetaxel, compare the efficacy on each cell line of combinations, including docetaxel alone and various combinations of rapamycin and trehalose.

[4] See McGarty, Autophagy and Cancer Sept 2018, https://www.researchgate.net/publication/327585718_Autophagy_and_Cancer?_sg=HZXIvqsGMCyBPt2XDHcBkSUmRKhyXxUL9c3OV014LqvHqchk3i8SJqriqQB0uV2WVjcoWKjuS6MTlUP4W3TtMdVNDtWfMgZWz7dAGhAS.7UTQULybvAr9cqEf6tx0O1ovz_kMlFNjCDz7y7ivWAnnHQGh8gZWMWA3Wb3-GOB16M35EaK5R65izu3qYdY26w

The authors note in their paper:

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.[5]

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.

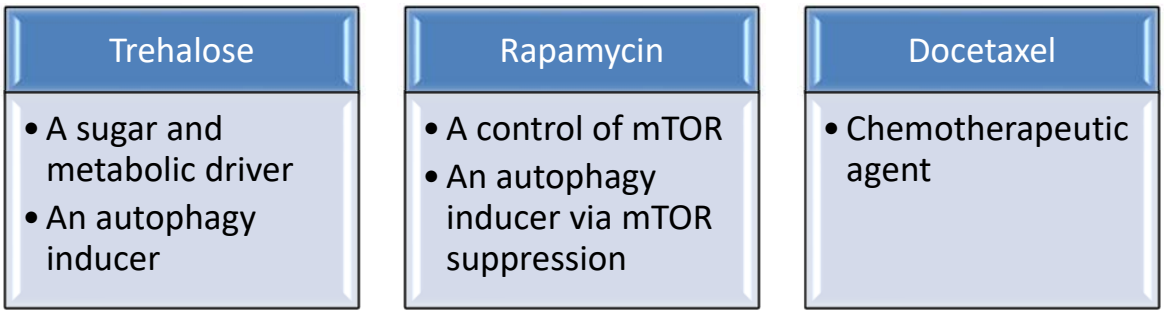
In PC3 cells, rapamycin retained its ability to activate autophagy without evidences of mitophagy even in presence of docetaxel.

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.

The three putatively therapeutic approaches are:

[5] See Wu et al for a discussion of these cell lines.



Now docetaxel is a taxane therapeutic from the genus Taxus. It works as follows (a description of paclitaxel as similar therapeutic)[6]:

Microtubules are composed of polymers of tubulin in dynamic equilibrium with tubulin heterodimers composed of alpha and beta protein subunits. Although their principal function is the formation of the mitotic spindle during cell division, microtubules are also involved in many vital interphase functions, including the maintenance of shape, motility, signal transmission, and intracellular transport. Unlike other antimicrotubule drugs, such as vinca alkaloids, which induce the disassembly of microtubules, paclitaxel promotes the polymerization of tubulin.

Now let us see if we can interpret this result in a systems context. The authors were looking at phenomenological results, namely experimentally derive drivers and responses. We alternatively want to see how we can then place this "bench detected" phenomenon into a system element.

The results presented are for three of the standard PCa cell lines. Specifically; LNCaP, PC3, and DU145. Each has a specific characteristic we review latter.

The results from the trial are shown below, as modified, for the DU145 PCa cell lines. (see Fig 8g where authors note: *DU145 cells were treated with trehalose 100mM or rapamycin 100 nM in combination with 20 nM docetaxel for 48 h and one-way ANOVA followed by Bonferroni post-test for drugs combination experiments*):

[6] See Rowinsky and Donehower

Survival Result	***100%***	***100%***	***80%***	***20%***		***20%***	***20%***	
Trehalose	0	1	0	0	1	1	0	1
Rapamycin	0	0	1	0	1	0	1	1
Docetaxel	0	0	0	1	0	1	1	1

Note that docetaxel works in all cases, with or without the other two elements under consideration. Moreover, the results seem identical no matter we see the use of one, the other, or not of the alleged adjuvants.

In contrast they show for the LNCaP PCa cell line the following result.

Survival Result	***100%***	***90%***	***80%***	***60%***		***80%***	***30%***	
Trehalose	0	1	0	0	1	1	0	1
Rapamycin	0	0	1	0	1	0	1	1
Docetaxel	0	0	0	1	0	1	1	1

In this cell line, the LNCaP line, the result is striking. Namely docetaxel plus rapamycin yields a significant growth suppression whereas the sugar with docetaxel does little. In addition docetaxel alone does show results but not near what we see with the use of rapamycin.

We should note that where the authors note: *LNCaP cells were treated with 1, 2, 10, 20, 50, 100 nM docetaxel. MTT assays were performed. Data are mean ± SD of six independent biological samples (n = 6). Each experiment was repeated three times. Statistical analysis of data was performed by Dunnet test (*p < 0.05). e LNCaP cells were treated with trehalose 100mM or rapamycin 100 nM in combination with 2 nM docetaxel for 48 h and one-way ANOVA followed by Bonferroni post-test for drugs combination experiments (*p < 0.05). f DU145 cells were treated with 1, 10, 20, 50, 100 nM docetaxel. MTT assays were performed. Data are mean ± SD of six independent biological samples (n = 6). Each experiment was repeated three times. Statistical analysis of data was performed by Dunnet test (*p < 0.05).*

Thus the results are materially different between the two PCa lines, namely DU415 and LNCaP.

Finally for the PC3 cells the authors note (as edited in an attempt for some clarity):

*Docetaxel treatment **increased** the number of apoptotic PC3 cells by about 40% (from 9.46 to 13.64%)*

Which we infer means that one saw that originally 9.46% of the PC3 cells were apoptotic and when treated with docetaxel the number increased to 13.64% apoptotic. Thus from the interpretation of the language used we see a change or increase in apoptosis of 40% , not an overall change in survival.

*and trehalose cotreatment **reduced** apoptotic PC3 cells by about 30% (from 13.64 to 9.48%)*

Now we see it started with the 13.64% which we may infer was what was remaining from the treated docetaxel cells alone and now inferring that the same PC3 cell line when treated with docetaxel and trehalose actually saw survival increase or apoptosis decrease from the 13.64% to 9.48%. But, if we may infer from the first part of the sentence, 9.46% was the apoptotic rate when there was no treatment! One must also try to understand the precision noted with the techniques employed, and asks if it warrants such precision. There is no questioning here just a need for clarification.

*and **drastically reduced** early apoptotic PC3 cells (50% from 10.86 to 5.73%(sic)).*

As expected

*rapamycin cotreatment **did not alter apoptotic PC3 cells induced by rapamycin alone** (10.75 compared to 10.11)*

From the above, one possibly can infer the following. The treatment with docetaxel and rapamycin resulted in 10.75% apoptosis whereas rapamycin alone resulted in 10.11% apoptosis.

even if it increased necrotic PC3 cells.

Moreover, in high-dose docetaxel treated PC3 cells trehalose reduced late apoptotic cells by about 45% (from 6.15 to 2.75%) (Fig. S4). These results are consistent with previous studies and shows that trehalose counteracts docetaxel-induced apoptosis.

This can be shown in the following comparable Table as above now for PC3 using the statements made as above.

Apoptosis	***9.6%***	***NA***	***10.11%***	***13.64%***		***9.48%***	***10.75%***	
Trehalose	0	1	0	0	1	1	0	1
Rapamycin	0	0	1	0	1	0	1	1
Docetaxel	0	0	0	1	0	1	1	1

The authors conclude:

Collectively, the results obtained lead to hypothize that trehalose-induced mitophagy represents a crucial cellular survival response involved in chemotherapy resistance.

Alternatively, activation of autophagy mediated by rapamycin is a phenomenon that causes cell death and enhances the effect of chemotherapy.

It is not clear from the above reported result that such a conclusion is warranted for the DU415 line but clearly for the LNCaP line. It seems problematic for the PC3 lines as well. The results seem to indicate that indeed mTOR suppressor rapamycin with docetaxel is the same as docetaxel alone.

In conclusion, our findings clarify that mitophagy is a key mechanism in docetaxel-resistance in CRPC and focus that the molecular mechanisms of autophagy activation are crucial for the therapeutic use of combination therapies.

They also note for the PC3 cells:

1. *Trehalose induces autophagy in PC3 cells*
2. *Rapamycin induces autophagy in PC3 cells*
3. *Docetaxel induces apoptosis, autophagy, and mitochondrial fission in PC3 cells*
4. *Trehalose and rapamycin differently modify mitochondria/autophagosomes colocalization in docetaxel-treated PC3 cells*
5. *Trehalose and rapamycin differently modify mitochondria/lysosomes colocalization in docetaxel-treated PC3 cells*
6. *Trehalose and rapamycin different(sic) counteract docetaxel induced apoptosis in PC3 cells*
7. *Opposite role of trehalose and rapamycin on docetaxel induced cell death in PC3 cells*

Thus both induce autophagy. We understand somewhat the reason what rapamycin does this since it inactivated the mTOR molecule. In a similar but less well explained reason for the sugar. These results although a bit conflicting present an intriguing study. Autophagy does at times appear to be a beneficial adjuvant when induced via mTOR. However it can also be said that autophagy induced otherwise has a de minimis effect.

3 THE CELL LINES

As is common in many cancer research efforts, in vitro standardized cell lines are employed so as to allow for reproducibility and reference points. We discuss them here. It should be noted that in vitro cell lines have potentially substantial issue due to their lack of in vivo interfaces. Thus the results from a cell line would apply solely to that cell line and extension to a human environment would be highly questionable.

Wu et al have discussed these cell lines in detail. They note:

The most commonly used cell lines for prostate xenograft models are LNCaP, PC3 and DU145.

As we have noted previously these are the targets for the paper under analysis. They then continue to explain each.

3.1 LNCaP

The LNCaP cell line is androgen sensitive human PCa cell line derived from lymph node metastasis. LNCaP cells are known to have a ***mutated androgen receptor*** *(AR) (T877A). Although its tumorigenicity proved rather poor in athymic nude mice, the LNCaP cell line has been used to create LNCaP sublines which can be grown in vivo after subcutaneous or orthotopic inoculation., including LNCaP-Pro3-5 and LNCaP-LN3-4. Subsequent to implantation into the prostate, LNCaP-LN3 cells produced a higher incidence of regional lymph node metastases compared to LNCaP. After intrasplenic implantation, LNCaP-LN3 cells also yielded experimental liver metastases. The metastatic LNCaP-LN3 cells exhibited clonal karyotypic abnormalities, were less sensitive to androgen (in vitro and in vivo), and produced high levels of prostate-specific antigen (PSA).*

3.2 PC3

*The PC3 cell line was o****riginally derived from a bone metastasis of human prostatic*** *adenocarcinoma origin. Intravenous injection of PC3 has led to the establishment of lymph node metastases. … Some sublines from PC3 have been generated that have increased metastatic ability. PC-3M cells are metastasisderived variant of PC3.*

Tumors from the prostate or lymph nodes were harvested after intraprostate growth, and cells were reinjected into the prostate. This cycle was repeated three to five times to yield cell lines PC-3M-Pro4, and PC-3M-LN4. PC-3M-LN4 cells produced enhanced regional lymph node and distant organ metastasis. After i.v. or intracardiac inoculation, PC-3M-LN4 cells produced a higher incidence of lung metastasis and bone metastasis, respectively. As PC3 is negative for AR expression, PC3-AR, clonal PC3 cell line stably transfected with AR, has been used in various studies.

3.3 DU145

The DU145 cell line, which has ***less metastatic potential compared with PC3 cells****, was derived from a brain metastasis of human prostatic adenocarcinoma origin. PC3 and DU145 cells are androgen-independent PCa cells, however neither cell line express AR.*

Since most human androgen-independent PCa maintain AR expression, efforts have been focused on developing AR positive androgen-independent PCa cell lines. LNCaP-abl cell line was established by … in 1999 by culturing androgen-sensitive LNCaP cells in androgen-depleted medium for 87 passages. The LNCaP-abl cells express high levels of AR and displayed a hypersensitive biphasic proliferative response to androgen until passage.

Growth of LNCaP-abl xenografts in nude mice was stimulated by bicalutamide and repressed by testosterone. IL-6 reportedly has divergent effects on the growth of the androgen-responsive cell line LNCaP. By using prolonged treatment with this cytokine a subline, LNCaP-IL-6+, was generated that does not show the growth-inhibitory response in spite of upregulated expression of endogenous IL-6. LNCaP-IL-6+ cells grow more rapidly in nude mice than do their counterparts, LNCaP-IL-6-, which were established after serial passaging in the absence of IL-6 (Steiner, et al. 2003). In LNCaP-IL-6+ cells, there is an upregulation of cyclin-dependent kinase 2 and reduced expression of the tumor suppressors pRb and p27. … developed the androgen-independent subline, LNCaP C4-2. This LNCaP subline was able to metastasize to bone, however the frequency was low (2 of 20).

Sublines derived from C4-2, designated B2, B3, B4, and B5, were established and have a higher propensity to metastasize to bone and cause osteoblastic lesions. The LNCaP-AI cell line is an androgen independent prostatic carcinoma derived from the androgen-dependent LNCaP-FGC cells. LNCaP-AI cells express higher level of AR compared to LNCaP cells and retain sensitivity to androgen stimulation.

Thus the three cell lines used in the discussed experiment are somewhat standards but each has their specific characteristics.

3.4 SUMMARY

The following is from Wu et al as modified detailing the cell lines in use.

Cell line	*Advantage*	*Disadvantage*
LNCaP	LNCaP sublines can be grown in vivo LNCaP-LN3 cells produced a higher incidence of regional lymph node and liver metastases.	Mutated androgen receptor Tumorigenicity is poor in athymic nude mice
PC3	Derived from bone metastasis high metastatic potential	AR negative no response to androgens, glucocorticoids, or epidermal or fibroblast growth factors.

Cell line	*Advantage*	*Disadvantage*
DU145	Derived from brain metastasis AR negative Less metastatic potential than PC3	
C4-2B	Readily forms tumors in intact hosts Androgen independent growth	
MDA PCa 2a/2b	Double AR mutation: L701H and T877A Derived from bone metastases Androgen-sensitive Retain functional differentiation (express PSA, PAP)	Bax is positive in MDA PCa 2a cells but is negative in MDA PCa 2b cells
LAPC-4	Hormone-responsive Wild type AR	Can progress to androgen independent when grown in female or castrated male mice
VCaP	Wild type AR Derived from a vertebral bone metastasis of a hormone-refractory prostate tumor express PSA, prostatic acid phosphatase, cytokeratin-18	Difficult to study TMPRSS2-ERG rearrangement *in vitro* due to the presence of wild-type TMPRSS2 and ERG gene
LNCaP-abl	Express high level AR Basal AR transcriptional activity is 30-fold higher in LNCaP-abl than in LNCaP cells	
LNCaP-AI	AR positive at high level Express high level Bcl-2 p16 expression is reduced	
CWR22	Expresses PSA and AR. Growth is stimulated by epidermal growth factor	Slightly stimulated by DHT

What is essential to note is that these are all in vitro cell lines and it is well known that many of the extracellular factors such as the ECM and inter-cellular signalling play a critical role in PCa. We come back to this latter.

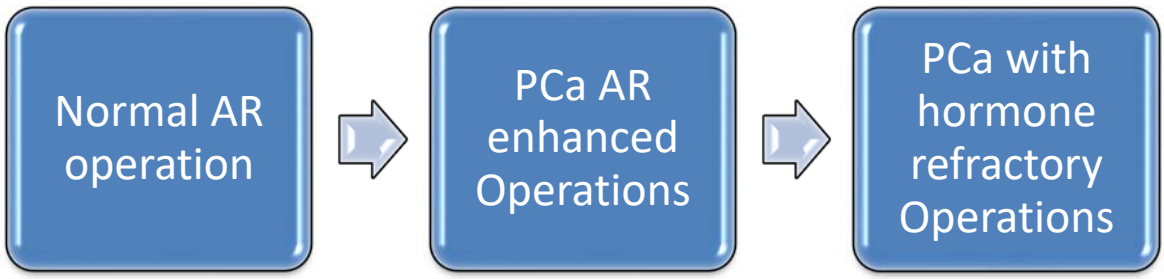
4 ANDROGEN RECEPTORS, PCa AND PROLIFERATION

We first briefly review the androgen receptor issue in Ca. The Androgen Receptor, AR, is located on Xq12. Androgens mediate a wide range of developmental and physiological responses and are especially important in male sexual differentiation and pubertal sexual maturation, the maintenance of spermatogenesis, and male gonadotropin regulation. The principle steroidal androgens, testosterone and its metabolite DHT (5-Alpha-Dihydrotestosterone), mediate their biological effects predominantly through binding to the AR (Androgen Receptor), an androgen-inducible member of the nuclear receptor super-family of transcription factors.

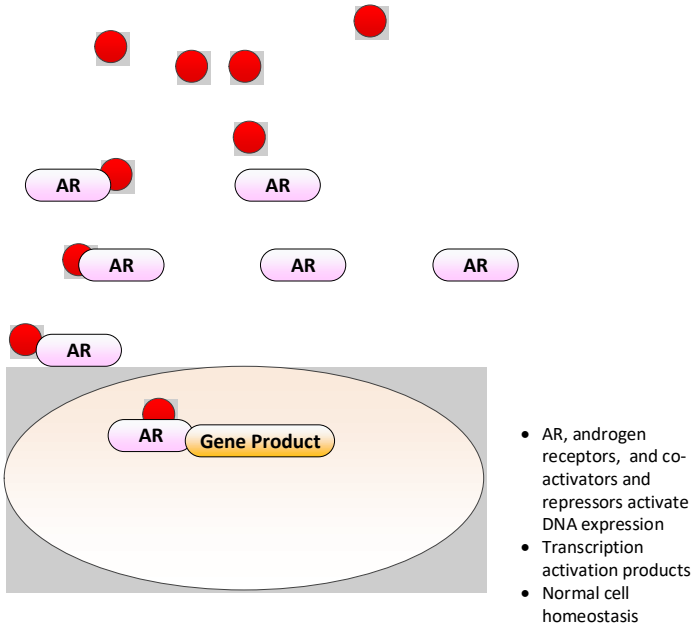
The normal function of the Androgen Receptor is as follows:

1. Testosterone enters the cell
2. If 5-α-Reductase is present the testosterone is converted to dihydrotestosterone, DHT.
3. The DHT then binds with the AR and the entity undergoes a transformation and releases heat shock proteins, HSPS
4. Then there is a phosphorylation
5. The AR translocates to the nucleus where it dimerizes, and there is DNA binding.
6. Target genes are then transcribed.

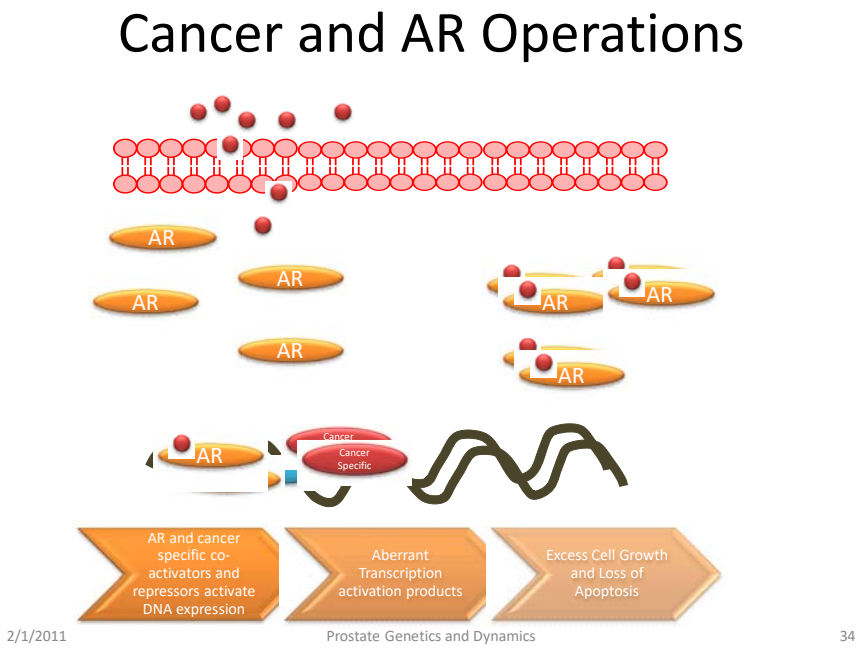
AR mediates transcription of proteins which are essential for normal development. However as PCa progresses there is at first normal AR operation, then it is enhanced, and then the PCa which was dependent upon the AR function can become independent of it altogether. We depict that process below.



In normal AR operations, we show below the Testosterone coming into the cell and then it binds with the AR. It is this normal bonding which gives the AR the ability to manage a significant portion of the normal growth of the prostate cell. We use the graphics from Turner (2010) as modified below:



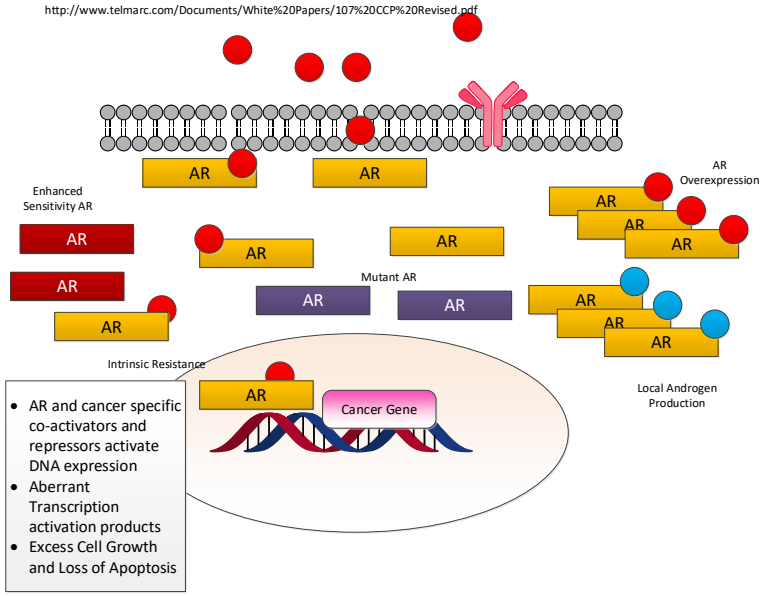
In the case of PCa we see the AR playing the role of excess growth enhancer.



As is best understood, the progression towards AR resistant PCa follows the path shown below.

Enhanced AR Sensitivity	•The reduced androgens interact with increased ARs so that the efficiency is increased
AR Mutations	•The ARs are mutated so that they are more efficient
Alternative Signalling Cascades	•The signalling channels on the cells surface may be modified
AR Overexpression	•The ARs may have an ability to excessively produce excess receptors
Local Androgen Expression	•Local internal cytoplasmic androgen production may occur
Intrinsic Resistance	•Cancer cells may be those with low intrinsic resistance

When the cell becomes refractory to AR functions, there may at first be AR overexpression and then a set of PCa specific receptors develop which result in metastatic grown as depicted below.



The recent work by Niu et al and that of Vis and Schroder describe in detail many of the specifics of the operation of the AR as currently understood. As regards to some details on specific pathway expressions the work of Nantermet et al (2004) states:

The androgen receptor (AR), when complexed with 5-dihydrotestosterone (DHT), supports the survival and proliferation of prostate cells, a process critical for normal development, benign prostatic hypertrophy, and tumorigenesis. However, the androgen-responsive genetic pathways that control prostate cell division and differentiation are largely unknown.

To identify such pathways, we examined gene expression in the ventral prostate 6 and 24 h after DHT administration to androgen- depleted rats. 234 transcripts were expressed significantly differently from controls ($p < 0.05$) at both time points and were subjected to extensive data mining. Functional clustering of the data reveals that the majority of these genes can be classified as participating in induction of secretory activity, metabolic activation, and intracellular signaling/signal transduction, indicating that AR rapidly modulates the expression of genes involved in proliferation and differentiation in the prostate.

Notably AR represses the expression of several key cell cycle inhibitors, while modulating members of the ***wnt and notch signaling pathways****, multiple growth factors, and peptide hormone signaling systems, and genes involved in MAP kinase and calcium signaling. Analysis of these data also suggested that* ***p53 activity is negatively*** *regulated by AR activation even though p53 RNA was unchanged. Experiments in LNCaP prostate cancer cells reveal that AR*

inhibits p53 protein accumulation *in the nucleus, providing a post-transcriptional mechanism by which androgens control prostate cell growth and survival. In summary these data provide a comprehensive view of the earliest events in AR-mediated prostate cell proliferation in vivo, and suggest that nuclear exclusion of p53 is a critical step in prostate growth.*

The authors continue:

AR induces cell proliferation and apoptosis in part because of its effects on cell-cell communication, particularly the stromal-epithelial interaction . As expected, the insulin-like growth factor (IGF-1) signaling system, which plays an essential role in prostate growth, was regulated at the level of ligand (IGF-1 was induced), extracellular binding protein (IGF-BP3 was repressed), and receptor (the IGF-1 receptor-1 exhibited biphasic expression). Also as expected, epidermal growth factor, which is induced by androgens in the prostate epithelium was upregulated . In addition to these well studied factors, several genes with potentially novel roles in the prostate were identified .

These include the transforming growth factor-2 (TGF-2) secretory partner latent TGF-binding protein- 1 (Ltbp1), which was repressed. Although the role of TGF-proteins in growth repression has been documented, latent TGF-binding protein 1 function in the prostate has not been extensively studied, although its expression might be frequently reduced in PCa. DHT also repressed granulin/epithelin (Grn), a cysteine-rich growth factor expressed throughout the reproductive tract that regulates growth in multiple epithelial cell types.

Given the role of Grn in certain epithelial neoplasias, it would be interesting to examine its expression in PCa. Finally, DHT led to the down-regulation of ephrin-A1/B61 (Efna1), a ligand for the Eph receptors expressed in various epithelia. EFNA1 regulates cell growth and inhibits tumor angiogenesis; its function in the prostate is unknown.

In short, other than p53 suppression, and a collection of other genes, there is not significant addition to what is already known. The Chen and Sawyers discussion ends with:

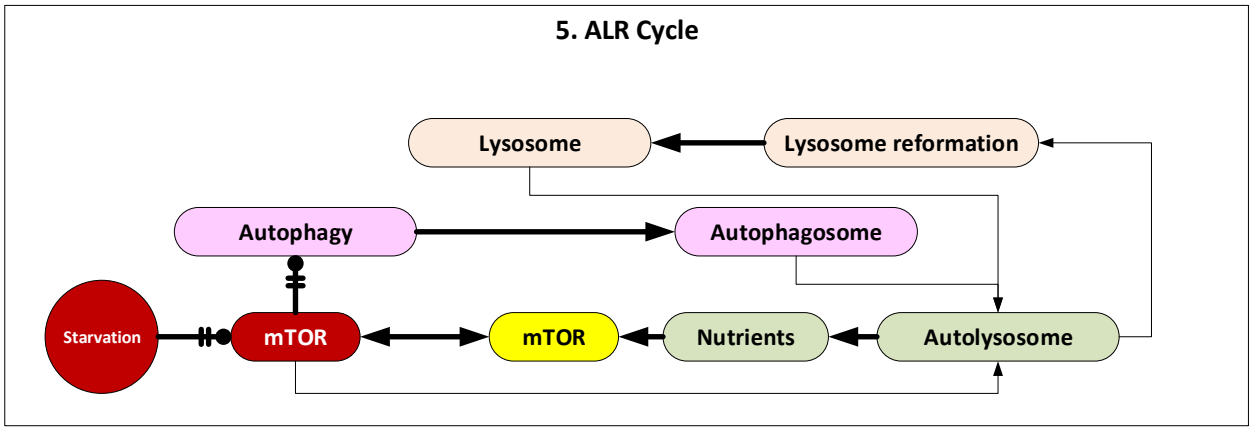
Despite the fact that AR occupies such a central role in prostate physiology and pathology, there ***is little insight into the direct AR target genes responsible for disease progression****. One very intriguing possibility, based on the high frequency of TMPRSS2-ETS fusion, is that the primary effect of antiandrogen therapy is to reduce expression of this presumed oncogene. Even though AR is overexpressed in more advanced stages of prostate cancer, recent profiling studies indicate that many AR target genes are actually expressed at lower levels in high-grade and metastatic lesions.*

This finding is consistent with older data that cancers with a high Gleason grade often produce lower levels of PSA and other markers of differentiation. Therefore, the relationship between the AR pathway and castrate resistance may also reflect the differentiation state of late-stage tumors. A small subset of very aggressive prostate cancers (small cell variant) does not express AR.

Clearly the importance of the AR is critical in PCa as it progresses and yet as noted above the full pathway development is still lacking.

5 AUTOPHAGY AGAIN

Autophagy is the process whereby a cell initiates to collection of and the processing and expulsion of certain targeted molecules.

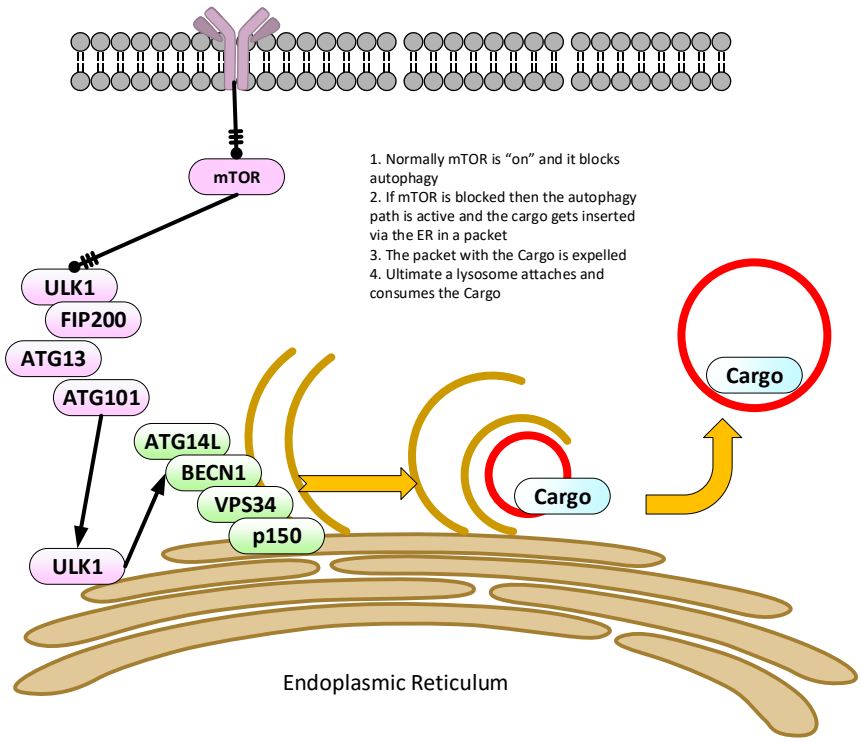


As Jung et al have noted:

Nutrient starvation, stress, or reduced availability of growth factors alarms eukaryotic cells to adjust metabolism to survive. An early response of the cellular metabolic adjustments involves inhibition of growth and induction of macroautophagy (referred to as autophagy unless otherwise specified) to optimize the usage of limited energy supplies. Autophagy, as a cellular process mobilizing intracellular nutrient resource, plays an important role in contributing to survival during these growth unfavorable conditions.

Eukaryotic cells have developed a mechanism through which autophagy induction is tightly coupled to the regulation of cell growth. Among the numerous components involved in the regulation of autophagy and growth, TOR is a key component that coordinately regulates the balance between growth and autophagy in response to cellular physiological conditions and environmental stress. Despite the significant progress in the autophagy study field, the mechanism by which TOR regulates autophagy remains not clearly understood. Here, we review recent findings that have made important progress in our understanding of the molecular link between TOR and autophagy in yeast and mammalian cells.

From Eberhart et al in Hayat the following is an example of autophagy.



mTOR is the key player. It can be activated and deactivated. The above is a description of the autophagy process, the internal "black box" of that process. The question we may ask is; where does it begin? I would argue just after mTOR control. Others may argue otherwise. Then we can ask also: where does it end? The answer is when the lysosome is used to lyse the contents. The boundaries between that are the autophagy "black box" process.

As Kondo et al have noted:

Autophagy is a process in which subcellular membranes undergo dynamic morphological changes that lead to the degradation of cellular proteins and cytoplasmic organelles. This process is an important cellular response to stress or starvation.

Many studies have shed light on the importance of autophagy in cancer, but it is still unclear whether autophagy suppresses tumorigenesis or provides cancer cells with a rescue mechanism under unfavourable conditions.

What is the present state of our knowledge about the role of autophagy in cancer development, and in response to therapy? And how can the autophagic process be manipulated to improve anticancer therapeutics?

They then proceed to detail some of the issues:

1. *Autophagy is a process that describes the degradation and recycling of proteins and intracellular components in response to starvation or stress.*

2. *At the early stage of tumour development, autophagy functions as a tumour suppressor. Expression of beclin 1 (BECN1), a mammalian orthologue of the yeast autophagy-related gene Atg6, reduces tumorigenic capacity through induction of autophagy. Mice that are Becn1+/− display a remarkable increase in the incidence of lung cancer, hepatocellular carcinoma and lymphoma.*

3. *At advanced stages of tumour development, autophagy promotes tumour progression. The tumour cells that are located in the central area of the tumour mass undergo autophagy to survive low-oxygen and low-nutrient conditions.*

4. *Autophagy protects some cancer cells against anticancer treatments by blocking the apoptotic pathway ('protective autophagy'). By contrast, other cancer cells undergo autophagic cell death after cancer therapies.*

5. *Autophagy is induced mainly through the phosphatidylinositol 3-phosphate kinase (PI3K)–AKT–mTOR (mammalian target of rapamycin) signalling pathway.*

6. *Manipulation of autophagy has the potential to improve anticancer therapeutics. When tumour cells induce protective autophagy, inhibition of autophagy could sensitize tumour cells to the treatment by activating apoptosis. On the other hand, induction of autophagic cell death can also have a therapeutic value.*

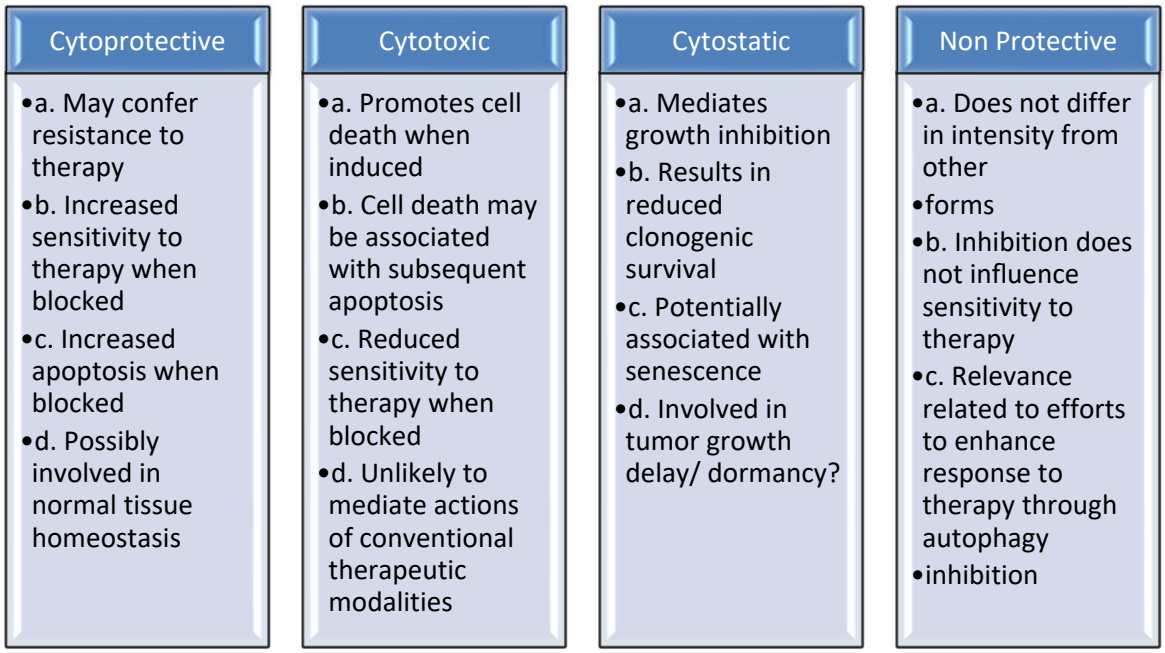
As Jiang and Mizushima note:

Autophagy is a major intracellular degradative process that delivers cytoplasmic materials to the lysosome for degradation. Since the discovery of autophagy-related (Atg) genes in the 1990s, there has been a proliferation of studies on the physiological and pathological roles of autophagy in a variety of autophagy knockout models.

However, direct evidence of the connections between ATG gene dysfunction and human diseases has emerged only recently. There are an increasing number of reports showing that mutations in the ATG genes were identified in various human diseases such as neurodegenerative diseases, infectious diseases, and cancers. Here, we review the major advances in identification of mutations or polymorphisms of the ATG genes in human diseases. Current autophagy-modulating compounds in clinical trials are also summarized.

The ATG set has been studied extensively and as we have noted previously, ATG5 is a key player.

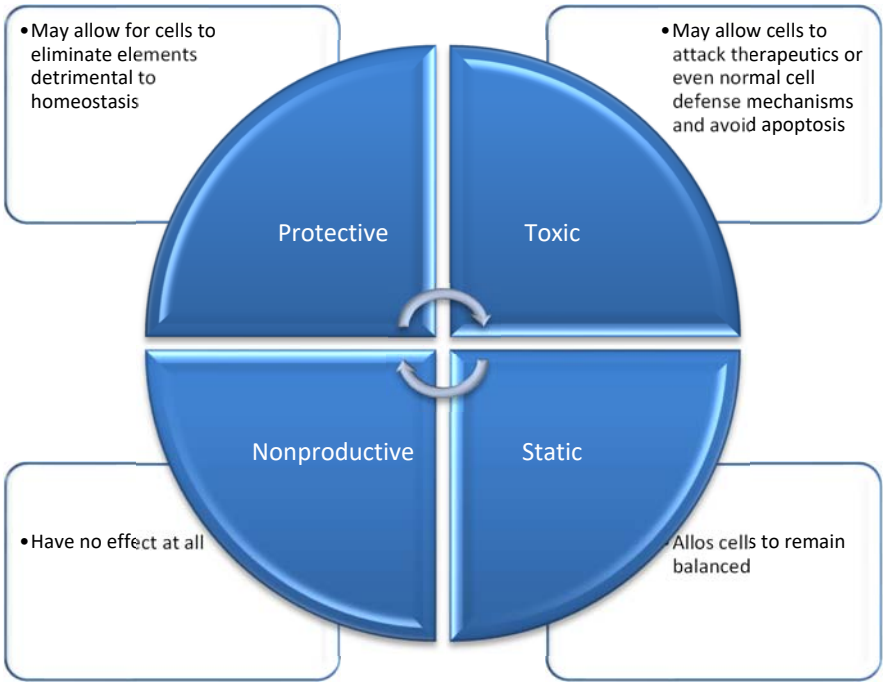
As Gerwitz notes and summarizes:



We further summarize this in the Table below.

Forms of autophagy	***Characteristics***
Cytoprotective	a. May confer resistance to therapy b. Increased sensitivity to therapy when blocked c. Increased apoptosis when blocked d. Possibly involved in normal tissue homeostasis
Cytotoxic	a. Promotes cell death when induced b. Cell death may be associated with subsequent apoptosis c. Reduced sensitivity to therapy when blocked d. Unlikely to mediate actions of conventional therapeutic modalities
Cytostatic	a. Mediates growth inhibition b. Results in reduced clonogenic survival c. Potentially associated with senescence d. Involved in tumor growth delay/ dormancy?
Nonprotective	a. Does not differ in intensity from other forms b. Inhibition does not influence sensitivity to therapy c. Relevance related to efforts to enhance response to therapy through autophagy inhibition

The four actions of autophagy are depicted below. Simply put, autophagy can be the "good, bad, ugly" of cellular functions. The question is; which one are we activating and why? There seems to be a limited amount of data on these issues.



Now Gerwirtz notes:

In a seminal paper by the Kroemer laboratory, the putative cytotoxic actions of autophagy for conventional antitumor drugs were largely laid to rest. These studies demonstrated that blocking autophagy induced by a host of therapeutic agents by knockdown of ATG7 did not result in protection from their antiproliferative or cytotoxic actions.

This finding, if it can be extrapolated to the clinical situation, would support the potential utility of autophagy inhibition as a therapeutic strategy if, in fact, conventional drugs (and possibly the hypoxic tumor environment) promote solely the protective form of autophagy. This strategy of blocking autophagy further assumes that autophagy is actually a consequence of therapy in human malignancies, which has not, to our knowledge, been proven. However, as also shown by

Kroemer's group in animal studies, tumor cells undergoing autophagy secrete factors that activate an immune response that is critical for drug effectiveness. Consequently, a pharmacologic approach that is actually effective in suppressing autophagy would be at best counterproductive and might actually interfere with the utility of conventional treatments by attenuating the immune response.

As Kim and Guan note regarding mTOR regulation and using it as a target:

mTOR promotes anabolic metabolism and inhibits autophagy induction.

Therefore, the regulation of autophagy with mTOR inhibitors provides a new therapeutic strategy for a variety of diseases, including neurodegenerative diseases, diabetes, and cancer.

Most available mTOR inhibitors that have been rigorously tested for clinical uses are rapamycin derivatives, and the majority of these tests have been focused on their anti-proliferation effects for cancer treatment. These compounds must be further evaluated in autophagy-related diseases such as neurodegeneration and cardiac myopathy, which are often associated with lysosomal and autophagy defects.

One critical factor that must be considered is the potential side effects of mTOR inhibitors. mTORKIs are cytotoxic, likely due to the inhibition of mTORC2, whereas rapamycin is generally cytostatic with less toxicity.

It might be advantageous to use mTOR-KIs for cancer treatment but not for chronic diseases such as neurodegeneration. Therefore, in the treatment of neurodegenerative or metabolic diseases, rapalogs are probably more desirable, as they have fewer side effects. Both rapalogs and mTOR-KIs have immunosuppressive effects that could also limit their potential application. Further pharmacokinetic studies are needed to determine the effective doses of mTOR inhibitors for inducing autophagy with minimal side effects.

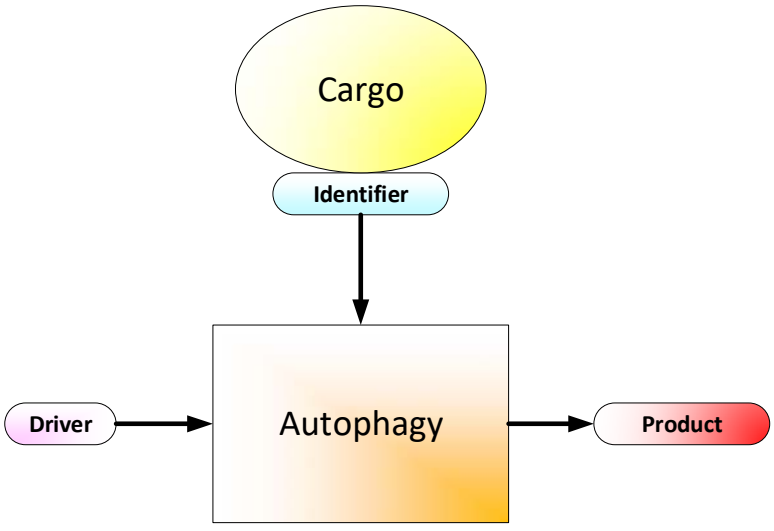
Thus we would investigate a full systems analysis of autophagy. Autophagy is a process, with three major elements.

1. Drivers: These are the signals that initiate autophagy.

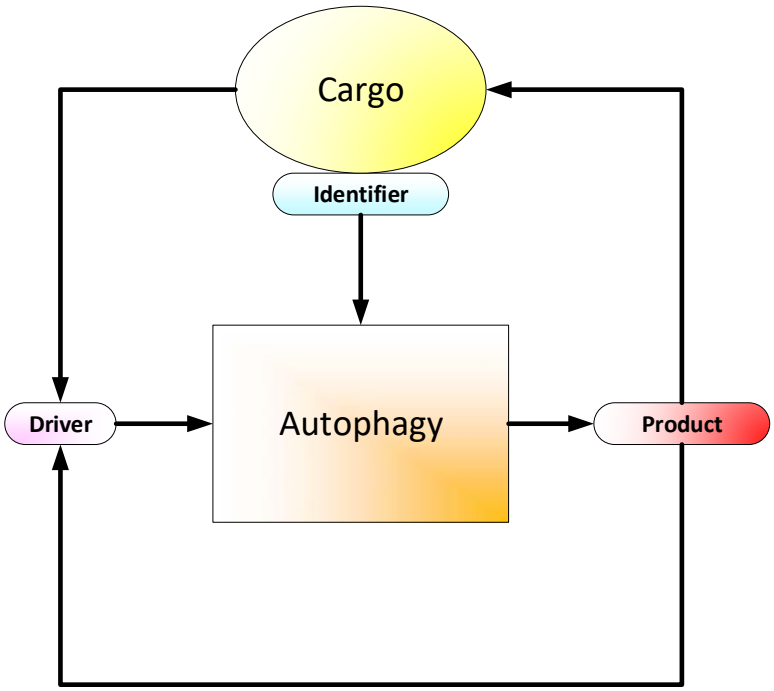
2. Identifiers: These are the characteristics of specific elements that identify them for degradation in autophagy.

3. Products: This is the result of the autophagy process.

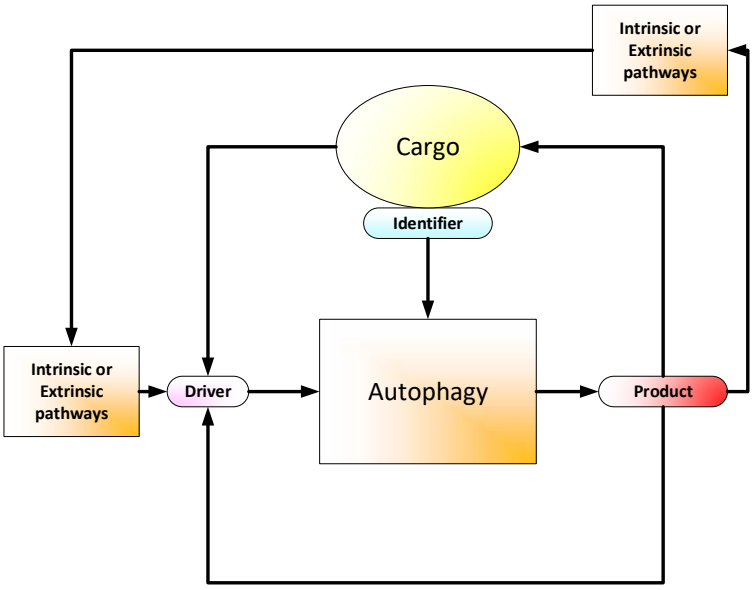
Now we look grossly at the system:



However there may be a multiplicity of feed back loops. This we depict below.



This, however, is just a beginning model. It may then be extended as follows:



Thus the challenge is to identify this system and look for control points. The target paper herein provides some insight to this process.

6 MTOR AGAIN

It is worth a high level review of mTOR as well since this gene product plays a critical role at the center of autophagy. Some of the original work was done by Sabatini and is recounted in detail in his JNAS paper in 2017. mTOR is a complicate control element at the heart of a cell. Active it can suppress autophagy, suppressed it allows autophagy to operate. Sabatini details the many control mechanisms of mTOR and also the many paths that mTOR then controls. Frankly the Sabatini paper is a critical summary to understand the details of this control element[7].

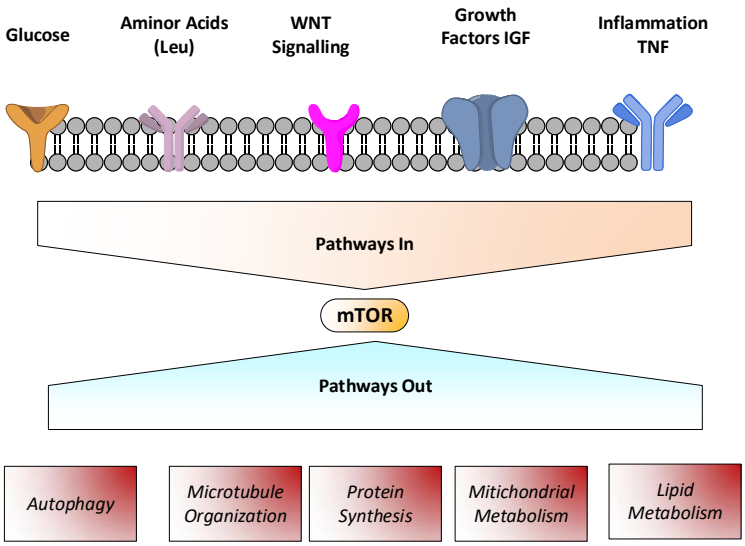
As NCBI notes[8]:

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.

This is an understatement. The short paper by Laplante and Sabatini is an excellent overview of mTOR. It has a complex set of inputs and a complex set of outputs. This paper provides and excellent and detailed map of how these function circa 2008. Specifically the author summarize:

mTORC1 integrates four major signals – growth factors, energy status, oxygen and amino acids – to regulate many processes that are involved in the promotion of cell growth.

These are the input drivers. The output is a primary goal of cellular growth.



[7] See http://sabatinilab.wi.mit.edu/indexDS.html for the details of the Sabatini Lab at Whitehead and MIT

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

The above is a simplified version of Laplante and Sabatini. Their characterization and detail is quite useful. The details regarding activation and suppression is also essential. Although the above demonstrates the key issues the details carry the complex sophistication in this gene product. An earlier paper by Sarbassov et al is also worth examining.

The paper by Guertin and Sabatini focuses on the role of mTOR in cancer. It is an excellent review work as of a decade ago. They conclude in their paper with:

Despite knowing about mTOR for nearly 15 years, we are just beginning to appreciate the complexity of the mTOR network. Since AKT activates mTORC1 by phosphorylating and inhibiting TSC1/2, and mTORC2 phosphorylates and activates AKT, mTOR may function both upstream and downstream of AKT. Defining these complex and perhaps cell-type-specific connections between mTORC1 and mTORC2 is an important challenge for the future. It is also becoming clear that the mTORC1 inhibitor rapamycin has an unforeseen capability to inhibit mTORC2, but only in a subset of cells.

The dual sensitivity of the mTORCs to rapamycin is particularly evident in endothelial cells, which is emphasized by the antiangiogenic property of rapamycin. Collectively, these findings are changing the view of the pathological role that mTOR plays in cancer and opening the door to new therapeutic strategies.

It is the complexity of the pathways that make mTOR attractive but complex. Furthermore Kinkaide et al have noted:

The AKT/mammalian target of rapamycin (AKT/mTOR) and ERK MAPK signaling pathways have been shown to cooperate in prostate cancer progression and the transition to androgen-independent disease. We have now tested the effects of combinatorial inhibition of these pathways on prostate tumorigenicity by performing preclinical studies using a genetically engineered mouse model of prostate cancer.

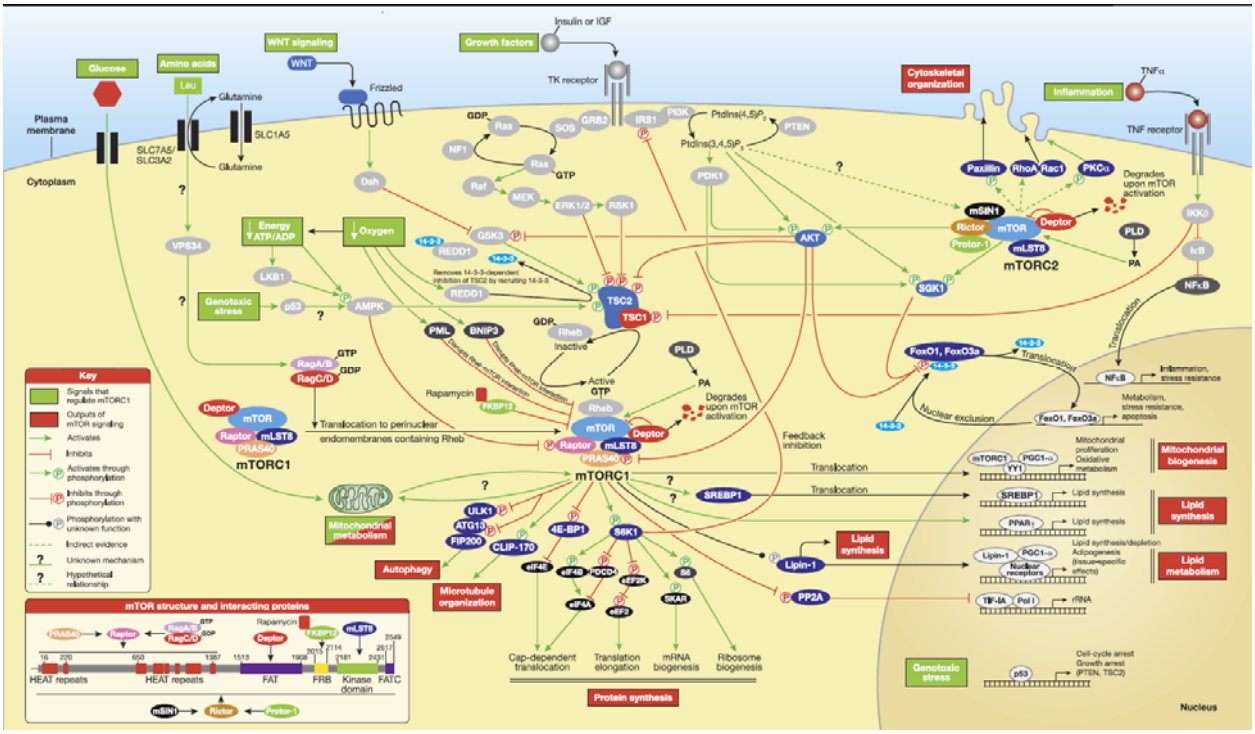
We report here that combination therapy using rapamycin, an inhibitor of mTOR, and PD0325901, an inhibitor of MAPK kinase 1 (MEK; the kinase directly upstream of ERK), inhibited cell growth in cultured prostate cancer cell lines and tumor growth particularly for androgen-independent prostate tumors in the mouse model. We further showed that such inhibition leads to inhibition of proliferation and upregulated expression of the apoptotic regulator Bcl-2–interacting mediator of cell death (Bim). Furthermore, analyses of human prostate cancer tissue microarrays demonstrated that AKT/mTOR and ERK MAPK signaling pathways are often coordinately deregulated during prostate cancer progression in humans.

We therefore propose that combination therapy targeting AKT/mTOR and ERK MAPK signaling pathways may be an effective treatment for patients with advanced prostate cancer, in particular those with hormone-refractory disease.

Now AKT and related kinases are upstream of mTOR but there are a multiplicity of feedback loops. As Hay has noted:

The downstream effector of PI3K, Akt, is frequently hyperactivated in human cancers. A critical downstream effector of Akt, which contributes to tumorigenesis, is mTOR. In the PI3K/Akt/mTOR pathway, Akt is flanked by two tumor suppressors: PTEN, acting as a brake upstream of Akt, and TSC1/TSC2 heterodimer, acting as a brake downstream of Akt and upstream of mTOR. In the absence of the TSC1/TSC2 brake, mTOR activity is unleashed to inhibit Akt via an inhibitory feedback mechanism. Two recent studies used mouse genetics to assess the roles of PTEN and TSC2 in cancer, underscoring the importance of Akt mTOR interplay for cancer progression and therapy.

In the paper by Laplante and Sabatini a while back they presented a map of mTOR signalling which we incorporate herein.



The above is clearly a key element in the development of a systems model.

7 CANCER IMPLICATIONS

The relationship between autophagy and cancer is complex to say the least. As we started in the Introduction to relate the efficacy of docetaxel in the case of PCa, we generally find an even more complex relationship. We refer to the work of Ravikumar et al in some detail. Although a bit dated it does present an exceptionally worthwhile discussion of the "on the one hand and on the other hand".

As Ravikumar et al note (as edited):

The role of autophagy in cancer is complex and highly debated.

On one hand, autophagy, as a housekeeping process capable of preventing accumulation of toxic cellular waste, some of which may be carcinogenic, can act as a tumor suppressor.

Indeed it may function to rid the cells of ROS elements. We generally understand what controls autophagy through mTOR. But what actually is the driver in the presence of mitogenic elements in general. How does the process obtain an initiator and how selective is it. Is autophagy just a clean-up process on detritus or a much more powerful process.

On the other hand, the ability of autophagy to support cell survival in conditions of hypoxia and nutrient deprivation may assist the survival of tumors, as these need to develop strategies to survive in sites where there is poor vasculature or reduced nutrient delivery to the core of a lesion. We will discuss both of these potential roles for autophagy in cancer.

This is the other hand side of the discussion.

Much circumstantial support exists for the concept that autophagy may act as a tumor-suppressor pathway. Many of the signaling pathways leading to tumorigenesis (e.g., upstream regulators in the mTOR signaling) overlap with those regulating autophagy. Furthermore, a number of autophagy genes such as Beclin 1, ATG5, ATG4c, and Bif-1 also have properties of tumor suppressors in mice.

We have discussed beclin1 extensively before and it is seen that its loss is carcinogenic. Can it be suppressed as well and if so by what and if so can it be reversed. If so what results.

Thus, for example, ATG4 knockout mice treated with a carcinogen have higher frequency of tumorigenesis compared with wild-type controls. Another argument in support of autophagy being protective against cancer is that the products of several tumor suppressor genes, such as DRAM, PTEN, DAPK, TSC1, and TSC2, positively regulate autophagy.

The same could also be true, at least in part, for the most frequently mutated in human cancers gene p53, though its role in autophagy remains controversial (see below). p53 can transactivate genes that induce autophagy, like DRAM and sestrin 1 and 2.

Conversely, oncogenes such as class I PI3K, Akt/PKB, and Bcl-2, among others, inhibit autophagy. Needless to say, the cellular functions of all these proteins are not limited to the regulation of autophagy, and therefore, this argument remains rather circumstantial. Autophagic activity is also reduced upon oncogenesis in murine pancreatic cancer models. Thus, in general, there is a positive correlation between molecules that induce autophagy and tumor suppression, and between molecules that inhibit autophagy and tumor progression.

As the cellular functions of these proteins are not limited to the regulation of autophagy, the case for their link with cancer is confounded by this caveat. The strongest support to date for the role of autophagy as an onco-suppressor pathway probably comes from the observations that autophagic genes like Beclin 1, UVRAG, and Bif-1 are mutated in human cancers, which presumably leads to the impairment of autophagic activity in mutant cells.

*The list may potentially be extended in the future to other autophagy genes, including ATG7 and ATG8 that are part of the loci frequently deleted in various types of tumors. The high penetrance of such mutations in human cancers (**Beclin 1, for example, is monoallelically deleted in 40–75% of sporadic human breast, ovarian, and prostate cancers**) suggests that impairment of autophagy might be an important step of tumorigenesis.*

Is beclin1 deleted or suppressed. One suspects that there is an absence of the gene product and not a deletion of the gene itself.

…It has also been reported that apoptosis-impaired MEFs treated with apoptosis inducers die of autophagy-dependent cell death. These initial observations were followed by numerous subsequent studies presenting similar findings both in vitro and in vivo. In agreement with this, radiation treatment as well as many potential anticancer drugs (including rapamycin, tamoxifen, arsenic trioxide, histone deacetylase inhibitors, etc.) are known to induce autophagy, possibly leading to autophagic cell death. However, the sole existence of cell death by autophagy has recently become a subject of intense criticism and urgently requires further clarifications, as discussed above.

This statement is compliant with the opening discussion.

Recently, White and colleagues proposed two alternative explanations for a role of autophagy as a tumor suppressor independent of its pro-death or pro-survival effects[9].

First, autophagy can prevent DNA damage, centrosome abnormalities, aneuplody, and chromosomal defects especially in metabolically stressed cells. This is likely to be due to the role of autophagy in the removal of damaged proteins and organelles, primarily mitochondria,

[9] Note that we will discuss White and her colleagues latter.

thereby preventing increases of ROS and hence further damage following genomic instability … tumor formation occurs in autophagy-deficient cells as a consequence of p62 accumulation, which enhances DNA damage by increasing the levels of ROS. Thus impaired autophagy can promote genomic instability leading to oncogenic activation and tumor progression.

Second, autophagy may also achieve its onco-protective role by preventing necrosis; impaired autophagy in metabolically stressed tumor cells that cannot die by apoptosis can enable death by necrosis, which is often associated with a chronic wound-healing response that is linked to tumor growth.

Another mechanism whereby autophagy may be protective against cancer is because it enables efficient cross-presentation of antigens. This process is important for the induction of adaptive immunity against cancer cells (and infectious agents).

While the idea of autophagy as a tumor suppressor process became well established over the past three decades and has been embraced by the majority of the scientific community, an equally impressive (if not more extensive) literature exists supporting the role of autophagy in the promotion of cancer.

The simplest possible reason for this might be that autophagy contributes to tumor survival by allowing cells to sustain themselves under conditions of nutrient deprivation, and tumor cells may partially rely on autophagy for energy production. Indeed, it has been found that despite an overall reduction of autophagic activity in various malignancies, autophagosomes remain present, especially in the most metabolically stressed regions of the tumor.

Metabolomic profiling of colon and gastric cancer tissues revealed very low glucose and high lactate and glycolytic intermediate concentrations, suggesting enhanced glycolysis and the Warburg effect. The clear accumulation of all amino acids except glutamine in the tumors was compatible with enhanced autophagic degradation of proteins and active glutamine breakdown for energy production, i.e., glutaminolysis. In this regard, it is interesting that Beclin 1, unlike many other tumor suppressor genes, has never been found to be biallelically mutated in malignant cells, suggesting certain dependence of tumors on autophagy.

In agreement with this, complete removal of Beclin 1 or Atg5 from cancer cells slows their proliferation and facilitates apoptotic cell death, suggesting a protective role for autophagy against apoptosis. Similarly, pharmacological inhibition of autophagy in colorectal tumors causes nutrient deprivation induced cell death, highlighting an essential role of autophagy for the survival of cancer cells. Another argument in favor of autophagy as a pro-tumorogenic process comes from the recent discovery that the reduction of p53 levels in many tumors can induce autophagy.

This finding is rather unexpected taking into account the earlier evidence in favor of p53 as a positive regulator of autophagy. Although the specific molecular mechanism of the negative regulatory effect of p53 on autophagy awaits further elucidation, this function of p53 seems to be independent of its transcriptional activity and is mediated by cytoplasmic, not nuclear, p53. It has been speculated that upregulation of autophagy in p53 mutant cells could potentially have

the advantage for cancerous cells, as autophagy may increase their resistance to apoptosis, possibly due to removal of pro-apoptotic mitochondria.

These and other findings have suggested that autophagy upregulation after chemo- and radiotherapy may be an attempt for self-preservation by malignant cells. Our current knowledge on the relationship between autophagy and cancer contains much apparently contradictory data, and sometimes, opposing interpretations of the same experimental evidence exist in scientific literature. This reflects an extreme diversity of various pathologies united under the common name cancer, varied experimental techniques used by the investigators, and different stages of the disease being studied. Taking into account the importance of the problem, untangling these contradictions should be prioritized.

A common theme, however, is beginning to emerge in the field. It is based firmly on the appreciation of autophagy as a tumor-suppressing process protecting the normal cell from the insults caused by the accumulation of unfolded, dysfunctional, aggregated proteins, increased levels of ROS, and DNA damage.

Thus functional autophagy is essential in preventing tumor initiation, and therefore, autophagy upregulation could potentially be exploited in prophylactic treatments. The prosurvival effects of autophagy could, however, also be exploited by existing tumors by allowing the transformed tissue to survive in conditions of hypoxia and undernourishment.

Thus reducing autophagy may be of benefit in existing tumors.

This has led to pharmacologically favorable inhibitors of autophagy, like chloroquine and its derivatives, being tested in clinical trials as sensitizers for radio- and chemotherapy in several malignancies.

The initial results of chloroquine treatment of patients diagnosed with glioblastoma multiforme confirmed some improvement of midterm survival and called for large-scale trial studies. Caution, however, is called for in interpreting the results of these and other clinical trials as all the drugs, either inhibitors or inducers of autophagy, tested to date have pleiotropic effects in humans, and it remains to be demonstrated whether the effect on autophagy is causative or merely an epiphenomenon.

The Cristofani et al conclusion is (as modified):

Acting as a defensive stress mechanism, autophagy is also involved in chemoresistance, by enhancing cell stress tolerance.

Moreover, autophagy by removing damaged mitochondria (mitophagy) prevents chemotherapy-induced apoptosis, since mitophagy increases apoptotic resistance.

Thus, the response to chemotherapy (docetaxel) may be modulated by autophagy inducers in CRPC cell lines.

*The **natural disaccharide trehalose is a potent autophagy inducer** used to improve the clearance of misfolded proteins causing proteotoxic cell stresses in cell and animal models of neurodegenerative diseases.*

However, it is unknown whether trehalose can activate autophagy in CRPC cells.

*The macrolide lactone **rapamycin is another autophagy activator**.*

*While **trehalose does not involve the mammalian target of rapamycin (mTOR)**, **rapamycin specifically inhibits the mTOR pathway**.*

The mTOR signalling represses autophagy and regulates cell growth, proliferation, survival, angiogenesis and is upregulated in almost 50% of PC.

In PC cells, rapamycin exerts cytotoxic effects and enhances radio- and chemiosensitivity.

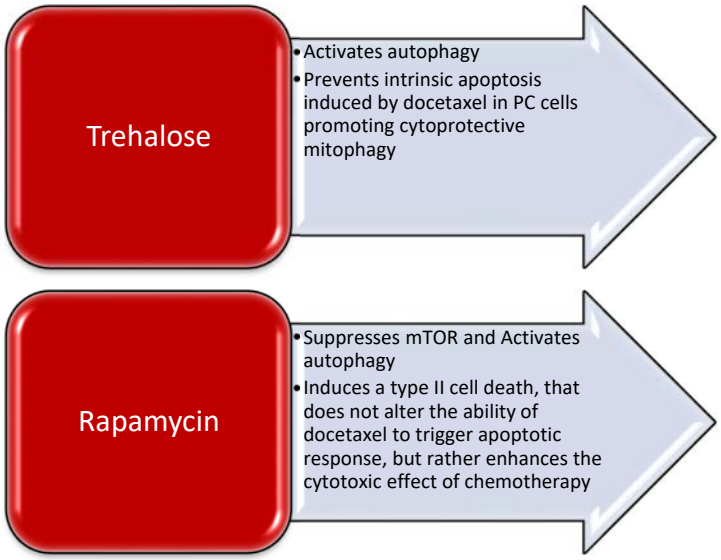
In this study, we investigated the effects of trehalose and rapamycin on the docetaxel response in classical PC cell lines ... demonstrating that these two autophagy activators exert very different roles on docetaxel-sensitivity.

Trehalose prevents intrinsic apoptosis induced by docetaxel in PC cells promoting cytoprotective mitophagy.

Conversely, rapamycin induces a type II cell death, that does not alter the ability of docetaxel to trigger apoptotic response, but rather enhances the cytotoxic effect of chemotherapy.

Thus, depending on the type of autophagy activation, docetaxel-induced toxicity may be differentially modulated in CRPC cells. These observations are crucial to design combination therapies to prevent cancer resistance and enhance the effects of anticancer therapies.

This study demonstrates a simple example of the theme, "on the one hand and on the other hand". These two elements, a glucose based sugar and rapamycin which affect differing pathways have substantially differing results.



The work by Cantor and Sabatini is a somewhat up to date presentation on cell metabolism and cancers. As they note:

The collection of advances made in our understanding of tumor metabolism in recent years has not only afforded a better understanding of the metabolic changes that help satisfy proliferative demands, but as critically, has revealed the diversity of mechanistic inputs and context-dependent determinants that can drive metabolic rewiring.

Moreover, numerous studies have illustrated that metabolic adaptations can in fact be selected for during transformation as well. Although the general uniformity of altered tumor metabolism lies in the shared ability of neoplasms to induce adaptations that stimulate rapid cell growth, we are now recognizing that the metabolic signature of cancer cells is one marked by the same complexities and diversity that characterize the disease as a whole. Namely, we are now beginning to unravel the heterogeneities that exist within the metabolic program of tumors that arise from different tissues, among different tissue subtypes, and even between cells populating a single tumor.

Our understanding of tumor metabolism continues to evolve as advances in several analytical technologies and modeling strategies are affording the implementation of systems level and integrated strategies for use in metabolic studies. Ultimately, these efforts will ideally facilitate further progress in capitalizing upon the exploitation of atypical metabolic features in cancer as a means of therapeutic intervention. Deciphering the interplay between genetic and nongenetic components that together contribute to metabolic reprogramming in a given setting may serve as the critical factor in determining therapeutic targets that enable maximal drug efficacy with minimal deleterious effect on normal cells

White and her students and researchers have done a great deal on autophagy and cancer[10]. We briefly examine some of Lab's results. Basically, as we have noted, autophagy can provide an energy source for cancer cell proliferation. In addition, it may also be a target for a therapeutic. Rapamycin and rapalogs have been used for a decade. Perhaps targeting combined with immune approach may be an effective mechanism.

For example, Kimmelman and White have recently noted:

Given autophagy's important role in tissue homeostasis, it is not surprising that the dysregulation of autophagy has been linked to multiple disease states, such as cancer and neurodegenerative disease. The role of autophagy in cancer has been of particular interest, and the work in this area has greatly expanded over the past several years.

Autophagy has a complex role in cancer, and its function can be dependent on biological factors, such as the tumor type, driving oncogene, and tumor suppressor gene constellation of a tumor, as well as technical aspects, such as the model system used to investigate its function. Initially, autophagy was thought to have a tumor-suppressive role. This was based on two major lines of evidence.

First, many of the activating mutations in oncogenes (e.g., PIK3CA) or inactivation of tumor suppressor genes (e.g., PTEN) would be predicted to inhibit autophagy.

Second, deletion of autophagy genes in the setting of certain mouse models can result in the initiation of neoplasia.

The initial identification of this phenotype was in Beclin1 (ATG6 ortholog) heterozygous mice, whereby these mice developed various neoplasms. An important aspect to note is that, in this case, autophagy was only partly attenuated, as a functional copy of Beclin1 was intact in the mice. In contrast, when ATG5 was completely deleted in a mosaic fashion in the whole mouse, thereby completely inhibiting autophagy in those cells with the deletion, the results were different. Interestingly, the only tissue that developed any neoplastic change was the liver, indicating that there are significant susceptibilities based on tissue type.

Additionally, the lesions that developed were benign liver tumors, which indicates that autophagy is required for progression to malignancy and explains why Beclin1 heterozygous mice with diminished but intact autophagy can develop malignant tumors. Similar results were obtained when Atg7 deletion was targeted to the liver.

The need for intact autophagy to progress to the malignant state may explain the apparent lack of mutations in canonical autophagy genes in human cancers. Beclin1 was initially identified as a haploinsufficient tumor suppressor gene, given that multiple breast and ovarian tumors

[10] http://cinj.org/research/white-laboratory-select-publications

demonstrated loss of one allele, although indication that these are passenger deletions, given its proximity to the BRCA1 tumor suppressor, has recently been suggested.

In contrast to its role in constraining tumor initiation, autophagy has been shown to have a critical pro-tumorigenic role in multiple cancer types. Initial studies demonstrated that autophagy was elevated in hypoxic regions of tumors, and that the process could promote tumor cell survival upon a variety of stressors such as nutrient and oxygen deprivation.

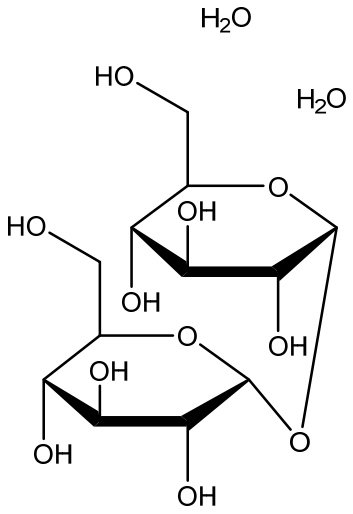
Autophagy is a process, not a single target. As a process it has inputs and outputs as well as a complex network in intra-process elements. Thus any attempt to control the process on the one hand argues for many possible control points yet on the other hand it argues for the complexity of a multiplicity of unknown and putatively unstable feedback loops.

8 THE CONTROLLERS

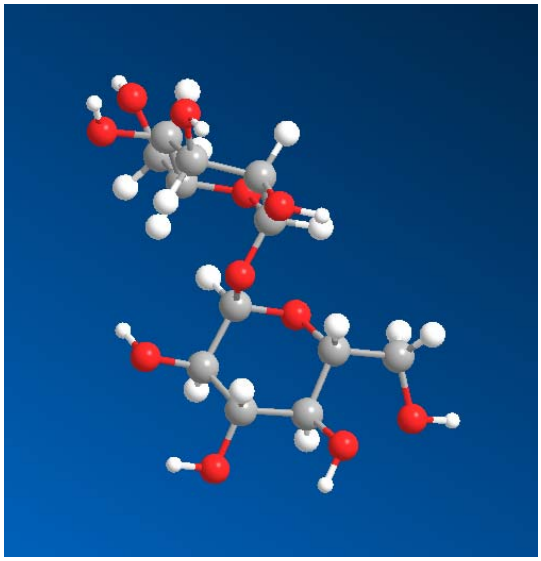
We briefly examine the three putative controllers here. Frankly it is a bit of a strange mix; a complex glucose molecule, rapamycin, a controller of mTOR, and docetaxel, an inhibitor of mitosis and thus of cell proliferation.

8.1 TREHALOSE

A sugar, trehalose, can induce non mTOR induced autophagy. The sugar is readily broken down to glucose by trehalase. The sugar is often found in organisms under stress such as plants and insects. It appears to be a configuration used to store glucose in a long term survival mode and used when stress requires glucose.



Its three dimensional forms is shown below.

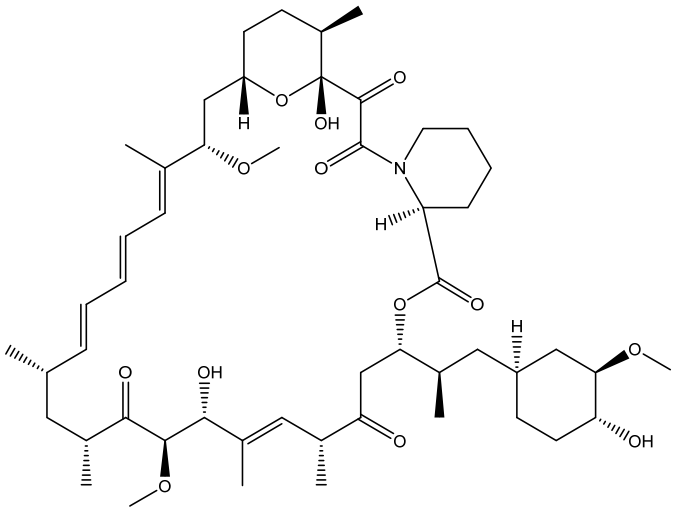


8.2 RAPAMYCIN

Rapamycin, also known as sirolimus, is a controller of mTOR. As Li et al note:

The mammalian target of rapamycin (mTOR) signaling pathway is a master regulator of cell growth and metabolism. Deregulation of the mTOR pathway has been implicated in a number of human diseases such as cancer, diabetes, obesity, neurological diseases and genetic disorders. Rapamycin, a specific inhibitor of mTOR, has been shown to be useful in the treatment of certain diseases. Here we discuss its mechanism of action and highlight recent findings regarding the effects and limitations of rapamycin monotherapy and the potential utility of combination therapy with rapamycin.

We depict the molecule below:



Li et al conclude:

Since the serendipitous discovery of rapamycin, considerable achievements have been made in understanding the mechanism of action and unraveling the intricate signaling network of mTOR. Uncontrolled mTORC1-mediated signaling is often observed in human diseases.

Therefore, it was thought that pharmacological inhibition of mTOR by rapamycin would have a substantial and wide range of clinical effects. Although rapamycin-based therapy has shown benefits for patients with RCC, TSC and LAM-related tumors, the use of rapamycin monotherapy in a broad spectrum of metabolic diseases, especially in treating cancers, is limited due to its modest efficacy. This may be explained by the inability of rapamycin to completely block mTORC1-mediated signaling events, the presence of several feedback loops and the up-regulation of compensatory pathways that promote cell survival and growth.

Thus, there is a critical need to further define these signaling processes and to develop new strategies that can overcome these drawbacks. The recent emergence of combination therapy with rapamycin may further increase efficacy and bypass feedback activation of survival pathways. Efforts that focus on exploring novel drug combinations with optimal doses will have great potential to yield an improvement of efficacy and safety profiles.

Additionally, significant promise remains for the discovery of new pathway inhibitors as well as the possibility that existing bioactives may directly or indirectly reduce mTORC1 and/or mTORC2 activity in monotherapy or in combination.

8.3 DOCETAXEL

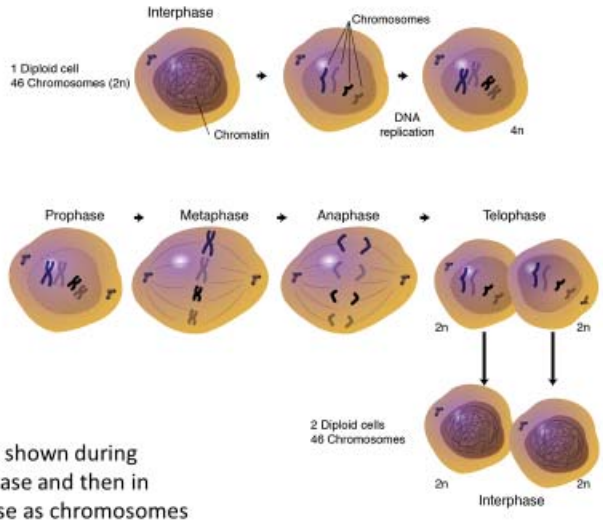
Docetaxel is a plant derived chemotherapeutic based upon the genus Taxus, a gymnosperm. From a classic paper by Rowinsky and Donehower we have a discussion of Paclitaxel, the first of these therapeutics:

The taxanes are an important new class of anticancer agents that exert their cytotoxic effects through a unique mechanism. Paclitaxel (Taxol), the first taxane in clinical trials, is active against a broad range of cancers that are generally considered to be refractory to conventional chemotherapy. This has led to the regulatory approval of paclitaxel in the United States and many other countries for use in the palliative therapy of patients with ovarian and breast cancers resistant to chemotherapy. The challenge now is to develop strategies using paclitaxel in the initial therapy of cancers in which cure or improved survival may be an achievable goal….

Microtubules are composed of polymers of tubulin in dynamic equilibrium with tubulin heterodimers composed of alpha and beta protein subunits. Although their principal function is the formation of the mitotic spindle during cell division, microtubules are also involved in many vital interphase functions, including the maintenance of shape, motility, signal transmission, and

intracellular transport. Unlike other antimicrotubule drugs, such as vinca alkaloids, which induce the disassembly of microtubules, paclitaxel promotes the polymerization of tubulin.

The tubules or microtubules are depicted in mitotic development below.



Tubules shown during metaphase and then in anaphase as chromosomes separate

The taxanes bind to the microtubules specifically the tubulin. They seem to bind preferentially to the tubulin in the assembled microtubules and inhibit the disassembly. This stops the mitotic process and kills the cell. Thus docetaxel inhibits the cell proliferation. It is not specific and does so to all proliferating cells and thus is a cause for other effects during chemotherapy. The specifics from the above referred to article continues:

At subnanomolar concentrations, paclitaxel inhibits the disassembly of microtubules, whereas it increases their mass and numbers at higher, albeit clinically achievable, concentrations. The microtubules formed in the presence of paclitaxel are extraordinarily stable and dysfunctional, thereby causing the death of the cell by disrupting the normal microtubule dynamics required for cell division and vital interphase processes. Paclitaxel also induces the expression of the gene for tumor necrosis factor a , but structure–activity studies indicate that these activities are not related to paclitaxel's effects on microtubule assembly, raising the issue of what part these cytokines play in the antitumor activity of paclitaxel. The binding site for paclitaxel is distinct from the binding sites for guanosine triphosphate, colchicine, vinblastine, and podofilox (podophyllotoxin).

Paclitaxel binds to the N-terminal 31 amino acids of the beta-tubulin subunit in the microtubule, rather than to tubulin dimers. In intact cells, paclitaxel induces the bundling of microtubules, which may be a useful clinical correlate of a lethal drug effect, and the formation of large numbers of asters of mitotic spindles It also enhances the cytotoxic effects of ionizing radiation

in vitro, possibly by inducing arrest in the premitotic G 2 and mitotic phases of the cell cycle, which are the most radiosensitive phases.

The feasibility of using paclitaxel in combination with radiation to treat patients with locally advanced lung, head and neck, and esophageal cancers, which are responsive to both kinds of treatment, is currently being evaluated.

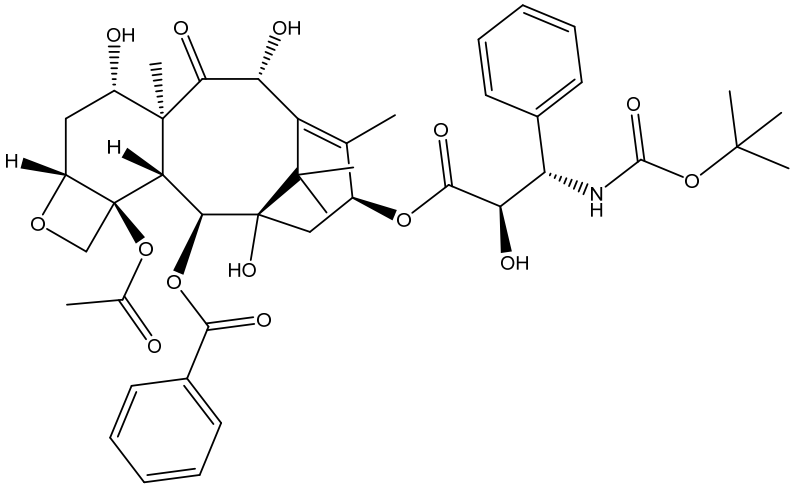
Two mechanisms of acquired resistance to the taxanes have been characterized.

First, some tumors contain alpha- and beta-tubulin with an impaired ability to polymerize into microtubules and have an inherently slow rate of microtubule assembly that is normalized by the taxanes.

A second mechanism involves the amplification of membrane phosphoglycoproteins…

Another approach to the development of the taxanes includes the identification of analogues through structure– function studies. One such analogue, docetaxel (Taxotere), is synthesized from 10-deacetylbaccatin III. As with paclitaxel, the principal toxic effect of docetaxel is neutropenia. Hypersensitivity reactions have also been noted, and premedication is now widely used. Unique toxic effects include a maculopapular rash with occasional bullous features, and with cumulative therapy, peripheral edema and pleural effusions that resemble a capillary-leak syndrome and often result in the discontinuation of treatment that function as drug-efflux pumps

The molecular structure of docetaxel is shown below.



9 OBSERVATIONS

This paper focused on two issues:

1. A better understanding of autophagy and prostate cancer treatment. Specifically, we know autophagy has a multiplicity of effects. It can be turned on and off via mTOR signalling as well as putatively other channels. Other cancer treatments such as the use of docetaxel can have their own effects and the question is; is there a synergy that is enhancing when using s therapeutic and at the same time activating autophagy?

2. An analysis of the Cristofani paper and its results related to the first issue. This is not a critique in any way of the Cristofani results as it is an attempt to analyze the wording and interpretation thereof. The author of this paper may have mis-interpreted some of the wording and as such this paper is a good faith analysis yet it also may be in error. Thus the author is open to any and all comments and corrections of the interpretations made herein.

As Singh et al have noted:

Evolutionarily conserved across eukaryotic cells, macroautophagy (herein autophagy) is an intracellular catabolic degradative process targeting damaged and superfluous cellular proteins, organelles, and other cytoplasmic components.

Mechanistically, it involves formation of double-membrane vesicles called autophagosomes that capture cytosolic cargo and deliver it to lysosomes, wherein the breakdown products are eventually recycled back to the cytoplasm.

Dysregulation of autophagy often results in various disease manifestations, including neurodegeneration, microbial infections, and cancer.

In the case of cancer, extensive attention has been devoted to understanding the paradoxical roles of autophagy in tumor suppression and tumor promotion.

In this review, while we summarize how this self-eating process is implicated at various stages of tumorigenesis, most importantly, we address the link between autophagy and hallmarks of cancer. This would eventually provide a better understanding of tumor dependence on autophagy. We also discuss how therapeutics targeting autophagy can counter various transformations involved in tumorigenesis.

Finally, this review will provide a novel insight into the mutational landscapes of autophagy-related genes in several human cancers, using genetic information collected from an array of cancers.

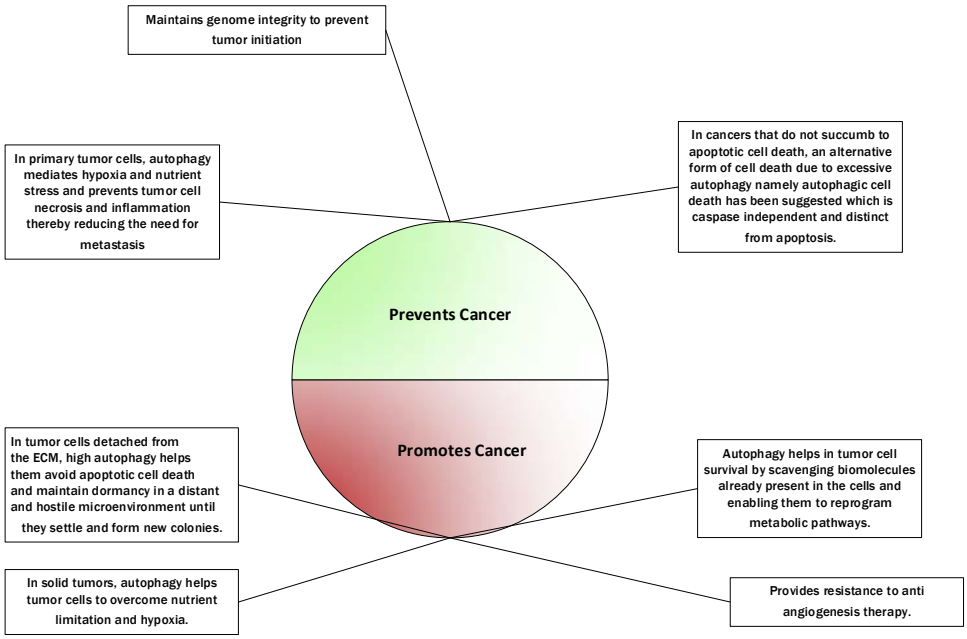
This recent work details the highly paradoxical operation of the autophagy system. It is paradoxical in the following ways:

Initiation: We all too often look at the mTOR only activator but we see that there are a multiplicity of ways in which this process is activated.

Targeting: The collection of "stuff" targeted to go into a autophagosome must have some identifying mechanism or otherwise we would be collecting every loose end. The process seems very selective in terms of what it targets and when it targets those items.

Pro/Anti Carcinogenic: Sometime it stops a malignant cell and others it expands such a cell. What mechanisms are involved?

Adjuvant Effects: It seems to be argued that autophagy can work with other therapeutics. No rationale is provided.



9.1 CELL LINES

The tests were performed on three of the most used cell lines. However the use of in vitro cell lines often suffers from a complexity of issues. Namely they are in vitro and thus suffer from the loss of environmental factors. Second they each have their advantages and disadvantages. Third the results may be specific solely to that cell line. It makes it easy to duplicate the result but consistency may hide the complexity of reality.

9.2 AUTOPHAGY LIMITATIONS

Just what does autophagy really do? We know what the taxane does to microtubules. We do not seem to reliably know however what happens to reduce the efficacy of the taxane use. Yet

extensive taxane usage is a classic "carpet bombing" approach since it inhibits all microtubules, from tumors to hair to immune cells. Yet we really do not have a good understanding of autophagy by itself no less as an adjunct to a taxane. What is the logic, what is the flow of control that this logic compels.

9.3 HUMAN FACTORS

In vivo analysis in critical. In vitro, albeit suggestive, is not dispositive. In vivo allows for the multiplicity of human interactions which are not present in specific cell lines. Understanding cancer means understanding the living human. Using homogeneous cell lines of mice in vitro may or may not tell us anything. Understanding the holistic patient environment, the unique individuality of each patient is essential.

The major human factor is that all cells have autophagy, most for beneficial purposes. Blocking all autophagy may very well have catastrophic effects. This is the classic problem of "carpet bombing" a patient with cancer. Event the most directive immunotherapeutic approaches are subject to this. Second, the problem of mitochondria diversity even in a single cell is an issue. As researchers examine the cells DNA, as we have noted herein, autophagy and mitochondrial effects can dominate especially in persistent proliferation, then it may be one or a few aberrant mitochondrial DNAs out of the thousands in a single cell. This issue may or may not be significant but it is factual.

9.4 STEM CELL ISSUES

We have been a continual observer of the cancer stem cell and cell of origin in PCa. Thus the issue is one of understanding whether using a cell line is even productive if one determines the stem cell. We leave this discussion to our previous work on the topic.

9.5 IMMUNE SYSTEM EFFECTS

There is an explosion of information regarding the use of the immune system in handling a variety of malignancies. PCa is very complex and thus far has evaded a direct attack as seen in such cancers as melanoma and hematological cancers. One then can wonder how autophagy plays a role here. Is autophagy an element in the immune systems operations? If so what and how does it do its functions?

As Kuballa et al have noted:

Stressors ranging from nutrient deprivation to immune signaling can induce the degradation of cytoplasmic material by a process known as autophagy. Increasingly, research on autophagy has begun to focus on its role in inflammation and the immune response. Autophagy acts as an immune effector that mediates pathogen clearance. The roles of autophagy bridge both the innate and adaptive immune systems and include functions in thymic selection, antigen presentation, promotion of lymphocyte homeostasis and survival, and regulation of cytokine

production. In this review, we discuss the mechanisms by which autophagy is regulated, as well as the functions of autophagy and autophagy proteins in immunity and inflammation.

Moreover Deretic et al note (as modified):

Autophagy as an immunological process can be organized in four principal manifestations …:

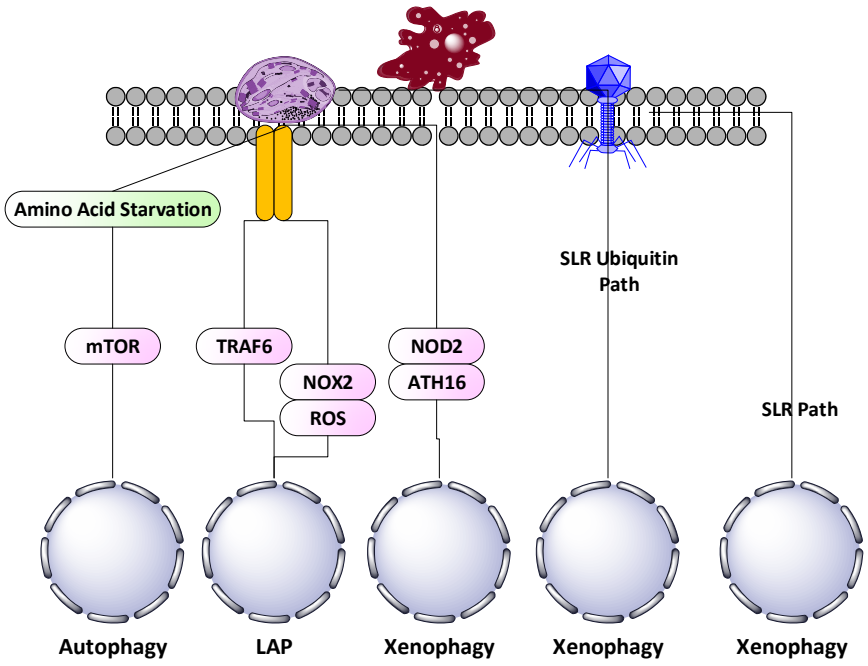
1. *direct elimination of microbes,*
2. *control of inflammation,*
3. *antigen presentation and lymphocyte homeostasis, and*
4. *secretion of immune mediators.*

Immunological autophagy fits but in some aspects exceeds the scope of autophagy as a cytoplasmic quality and quantity control process that also provides nutrients through cytosol autodigestion at times of starvation.

The word autophagy ("self-eating") refers to a collection of diverse processes enabling cells to digest their cytoplasmic constituents in lysosomes. This definition includes macroautophagy, microautophagy, chaperone mediated autophagy and noncanonical autophagy. Several non-autophagic roles, including immunological effects, of individual or subsets of autophagy factors have also been recognized.

In this review we cover only the sensu stricto autophagy – macroautophagy. We refer to it by name as autophagy and by function as a defined cell biological pathway that depends on specialized Atg factors. This pathway is distinct from other cytoplasmic digestive processes, including proteasomal degradation, by its ability to capture and eliminate large targets such as toxic protein aggregates, defunct or disused organelles, and invading microbes.

As an example they demonstrate the dealing with microorganisms.



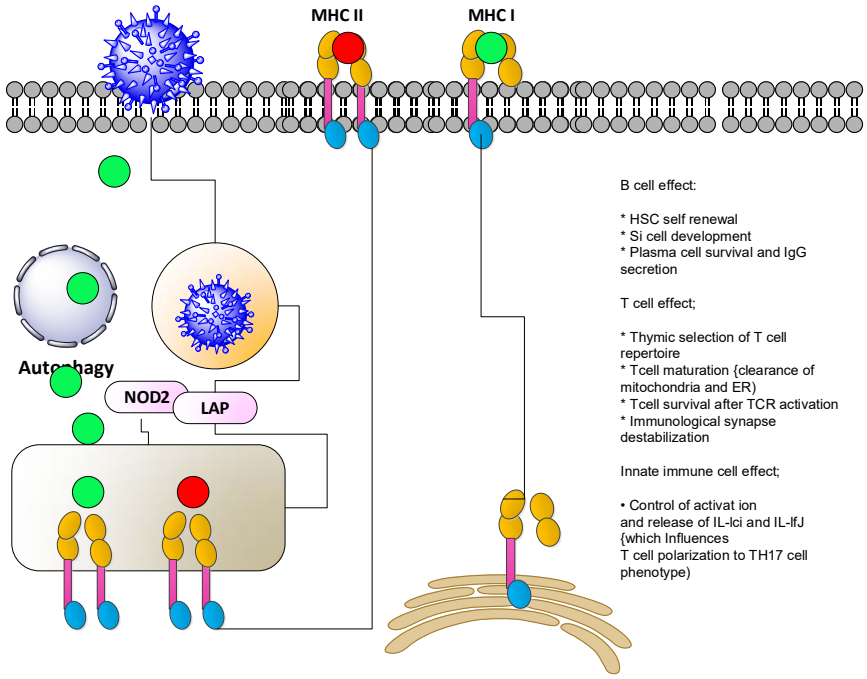
Deretic et al also note the situation for adaptive and innate immunity where they state:

The role of autophagy in adaptive immunity is shown (NOTE: we in this NOTE show this as modified below). Autophagy can increase the MHC class II presentation of cytoplasmic antigens, including self or viral antigens, as well as promoting the citrullination of antigens. LAP can enhance the processing of particulate antigens for MHC class II presentation. NOD2 enhances autophagic antigen presentation.

Autophagy may directly or indirectly affect MHC class I presentation by competing with the proteasome for substrates, by influencing the peptidome pools through the control of levels of components of microRNA (miRNA) machinery (for example, argonaute (AGO) and DICER), or by supporting unconventional MHC class I presentation.

In addition, autophagy affects the self-renewal of haematopoietic stem cells (HSCs), B1 cell development, plasma cell survival and IgG secretion. Autophagy affects T cell survival following T cell receptor (TCR) activation, and it destabilizes the immunological synapse. It also controls innate immune cell (such as macrophage) signalling through the release of interleukin-1α _(IL-1α) and IL-1β, which influence the polarization of T cells into T helper 17 (TH17) cells. Autophagy also affects naive T cell repertoire selection in the thymus and the survival and function of maturing T cells by removing the mitochondria and endoplasmic reticulum (ER), thus ensuring calcium homeostasis.

The system depicted in the above comment is shown below:



They conclude by saying:

Autophagy is a bona fide immunological process permeating many aspects of innate and adaptive immunity. Autophagy may have indeed evolved as one of the first antimicrobial defenses available to eukaryotic cells, shaped early on in evolution from what may have arisen as a metabolic and quality control pathway.

Autophagy has its own set of PRRs, the SLR adaptors, to eliminate invading microbes whereas pathogens have evolved strategies to evade autophagic capture. As evolution progressed, nearly all innate immunity systems such as conventional PRRs and inflammasomes have become integrated with autophagy. In the chordate lineage, this has further extended to adaptive immunity as best documented in mammalian systems.

In humans, a failure in parts of the autophagic apparatus can lead to inflammatory, autoimmune or general immunity disorders. The present knowledge of immunological autophagy is still in its infancy, and many interesting puzzles and important questions remain.

Thus clearly the nexus between autophagy and the immune system is significant. In fact many of the classic elements of the immune system, like Toll Like Receptors, are initiators of autophagy. The key question seems to be: is autophagy a process warranting study by immunologists as an adjunct to immunotherapy. Immunotherapy was generally passive. CAR-T cells changed that. Cane we expand it even further by employing the complex interplay with autophagy?

9.6 IS WARBURG AN ISSUE

We have a general understanding of the Warburg phenomenon[11]. Singh et al note:

In times of cellular stress, such as nutrient starvation, oxidative stress, hypoxia, or infection, autophagy plays a cytoprotective or an adaptive role. During nutrient starvation, autophagy breaks down macromolecules such as DNA/RNA, carbohydrates, proteins, and triglycerides. Hence, nucleosides, amino acids, sugars, and free fatty acids are then available for de novo synthesis of biomolecules or for generation of ATP to power cellular functions via the tricarboxylic acid (TCA) cycle and other metabolic processes

Thus perhaps there is a strong nexus between Warburg and autophagy. Singh continues:

Tumor cells adapt and change their metabolic pathways according to the microenvironment, which enables them to survive. In contrast to normal cells, tumor cells obtain energy by aerobic glycolysis rather than oxidative phosphorylation, a phenomenon termed as the "Warburg effect".

This adaptation eliminates the need of oxygen for ATP production . The pyruvate produced during glycolysis is converted to lactate, not acetyl CoA, hence causing a deficiency of TCA cycle substrates. This is an acquired characteristic of tumors as a result of mitochondrial impairment and it also helps them survive hypoxic conditions due to the lack of a proper blood supply. In the absence of pyruvates, cells will need other substrates to run the TCA cycle for ATP production, and autophagy can provide this by scavenging biomolecules already present in the cells. In RAS-driven cancers, cells utilize excessive glucose for glycolysis by increasing the expression of glucose transporters.

Cancers with RAS mutations are also addicted to glutamine as a substrate for the TCA cycle. Other amino acids generated by breakdown of proteins are also utilized in the liver for gluconeogenesis or for ATP synthesis via the TCA cycle. Fatty acids can produce energy by getting converted to acetyl CoA, and entering the TCA cycle. Autophagy also abrogates ROS toxicity due to damaged mitochondria.

The bipolar nature of autophagy in cancer. Autophagy has a complex and dual role in cancer. It maintains genomic integrity to prevent mutations that lead to tumorigenesis. In primary tumor cells, it prevents necrosis and metastasis. Excess autophagy can also be an alternate death mechanism in apoptosis-resistant cancers.

[11] See McGarty, https://www.researchgate.net/publication/322437754_Glucose_Warburg_Cancer_and_Pathways?_sg=J0tJ2Xi-Sp8KgujoUoiwst0yebCl2WCuUrWlBcJdVW0rSyjCu7MqLwRqH77Gx5NMAs7xOS-Ql6mZh5lCI8SzjIfUL_W4exdnGdyxs4Ri.epOAb1PcBy_Bx1h0xQmWI9ktINRKbm7E00PQO1vCQP8Mc572XkSAFwhM8s8PkPlKBLpdsVJQRXCLf02YdA_b5Q

In tumor cells detached from the extracellular matrix, autophagy prevents anoikis and enables their survival. It helps the tumor cells overcome lack of oxygen and nutrients, reprograms their metabolism, and provides resistance to anti-angiogenesis important in reprogramming metabolic pathways and enabling tumor cell survival.

Overall, autophagy is oncogenic at certain stages of tumor development and tumor-suppressive at other stages, and analysis of the role of autophagy in contribution towards several hallmarks of cancer will certainly provide insights into the realistic prospect of cancer therapy by modulating autophagy in a context-dependent manner

Thus the Warburg process details a mechanism whereby cancer and evade attack and proliferate in stressed environments. Autophagy may very well be an adjunct to that process providing the missing elements necessary for proliferation. The Warburg process is a means to get ATP in a less efficient manner. Autophagy is controlled externally by glucose. Thus the nexus is energy sources and uses. The question is; what are the details of this integrated process?

9.7 OF MICE AND MEN

One of the issues we face over and over is the murine model, especially the genetically engineered mouse models, "GEMM", and their relationship to human cancers. We have commented on this before and it is a complex issue. The mouse model has been helpful in isolating certain pathways and ascertain hematopoetic issues. However the human is highly complex and disparate. Thus we all too often find GEMM results at best suggestive and at worst confusing and misdirecting.

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11 APPENDIX: GENES INVOLVED IN AUTOPHAGY TYPE OF CANCER (FREQUENCY OF MUTATION)

Gene	*Colon*	*Breast*	*Pancreatic*	*Ovarian*	*Prostate*	*Lung*	*Liver*
ULKl	0.45%	0.72%	2.67%			2.08%	1.01%
ULK2	1.79 %	0.72%		0.32%		2.36%	3.03%
ULK3	1.35%	0.51%	1.33%			0.28%	
FIP200	3.59%	1.4 3 %	3.33%	2.53%	0.30%	4.31%	1.52%
ATG13	0.45%						
ATGlOl		0.31%	0.67%	0.32%			
WIPil	1.79%	0.41%	2.00%	0.90%	0.90%	0.97%	
WIPI2	0.90%	0.31%	1.33 %	0.63%		1. 11%	
PIK3R4	7. 17 %	1.13%	3.33%	1.58%	0.30%	4.72%	2.02%
PIK3C3	4.48%	0.82%	1.33%	0.32%	0.30%	2.92%	
WDFY3	14.80%	2.76%	6.00%	0.95%	1.51%	10.42 %	3.54%
WOR45	0.90 %	0.41%		0.32%		1.25 %	0 .51 %
ZFYVEI	2.69%	0.51%		0.32%	0.30%	2.36%	1.0 1%
BECNI	1.79%		1.33%	0.32%		0.83%	0.51%
AMBRAI	2.24%	1.74%	2.00%	0.32%	0.30%	1.81%	2.02%
BCL2	0.45%					1.39%	1.01%
RUBICON	1.79%	1.02%	4.00%	0.32%		2.78%	0.51%
RABSA			0.67%			0.42%	
ATG2A	3.59%	1.33 %	2.67%	0.63%	0.90%	3.33%	1.52%
ATG2B	4.93%	1.02%	4.00%	0.32%	0.60%	6.11%	3.03%
ATG3	0.45%	0.31%	0.67%	0.95%		0.97%	0.97%
ATG4A	1.79%	0.72%			0.30%	0.69%	
ATG4B	0.45%	0. 10 %				0.56%	1.01%
ATG4C	2.69%			0.32%	0.30%	0.56%	0.51%
ATG4D	2.24%	0.51%	0.67%		0.30%	1.25 %	0.5 1%
ATG2A	3.59%	1.33%	2.67%	0.63%	0.90%	3.33%	1.52%
ATG2B	4.93%	1.02%	4.00%	0.32%	0.60%	6.11%	3.03%
ATG3	0.45%	0.31%	0.67%	0.95%		0.97%	0.97%
ATG4A	1.79 %	0.72%			0.30%	0.69%	
ATG4B	0.45%	0. 10 %				0.56%	I.OJ%
ATG4C	2.69%			0.32%	0.30%	0.56%	0.51%

Gene	Colon	Breast	Pancreatic	Ovarian	Prostate	Lung	Liver
ATG4D	2.24%	0.51%	0.67%		0.30%	1.25 %	0 .51 %
ATG5	1.79 %	0.51%				0.56%	1.01%
ATG7	1.79 %	0.72%	2.00%			2.08%	
ATG9A	2.24%	0.61%	1.33 %			1.39 %	
ATG98	1.35 %	1.23%	0.67%	1.58%		2.08%	
ATG/0	0.45 %		0.67%			0.42%	1.01%
ATG12		0.10%			0.30%		
ATGl 6Ll	2.24%	0.72%				1.53%	0.51%
ATG16L2	0.45%	0.51%				0.97%	0.51%
MAPJLCJA	0.45%		1.33%			0.28%	
MAP JLC3 B						0.28%	
MAPJLCJC	0.45 %	0.10%	0.67%	0.32%		2.22%	
GABARAPLJ		0.20 %	0.67%	0.32%		0.14%	
GABARAPL2		0.20 %	0.67%	0.32%		0.14%	
RABJA		0.20%				0.14%	0.51%
RABI / A	0.90 %	0.31%	0.67%			0.14%	
RAB33B	0.90%				0.60%	0.28%	
NBRI	1.35 %	0.4 1%	0.67%			1.11%	2.53%
SQSTMJ	0.90 %	0.51%	2.67%	0.32%		0.97%	