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Modulation of Autophagy, a Therapeutic Approach to Mutated NRAS

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ABSTRACT

Here we discuss the mechanistic relationship of autophagy to apoptosis and specifically address the challenges in understanding the therapeutic approach to RAS mutated cancers, through modification of autophagy. We then present a case report of NRAS mutated positive liquid biopsy (on a patient with possible neuroendocrine tumor), and a treatment approach consisting of modulation of autophagy. We explain the mechanisms involved with tumor mutated allele frequency response in liquid biopsy and conclude that such approach could present as a viable option in RAS mutated tumor therapeutics and a basket trial recommended for future clinical validation.

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Background

Most of the studies performed about autophagy are focused on neurodegenerative diseases. Quite opposite to cancer, inhibition of apoptosis is a target for treatment of neurodegenerative disorders, as these category of diseases are caused by increased apoptosis and therefore anything that can inhibit the apoptosis enhances the disease regression, versus in cancer the apoptosis is desirable. So in one sense you can say that neurodegenerative disorders and cancer are two opposite sides of the same coin, when it comes to apoptosis. (and autophagy)

Autophagy, hallmarked by the formation of double-membrane bound organelles known as autophagosomes, is a lysosome-dependent pathway for protein degradation. The role of autophagy in carcinogenesis is context dependent. As a tumor-suppressing mechanism in early-stage carcinogenesis, autophagy inhibits inflammation and promotes genomic stability. Moreover, disruption of autophagy-related genes accelerates tumorigenesis in animals.

However, autophagy may also act as a pro-survival mechanism to protect cancer cells from various forms of cellular stress, after the tumor is formed- advanced stages. That said the existing literature is very ambiguous as whether autophagy suppresses tumorigenesis or provides cancer cells with a rescue mechanism under unfavorable conditions [1, 2].

Recently autophagy (as a non-apoptotic form of programmed cell death) has become a target for novel approaches in anticancer therapy. Indeed, cancer cells often present with mutations in the apoptotic machinery that result in resistance to most anticancer therapies and contribute to a relatively low response rate to therapies based on the use of pro-apoptotic strategies. Almost all chemotherapy agents induce autophagy and as such cause resistance to their own effect. (Macro-)autophagy however, itself can be a highly efficient mode of cell death induction by excessive self-digestion as demonstrated by experiments that studied the effect of radiation to induce autophagy cell death in apoptosis-deficient cells [3].

Necrosis is an irreversible inflammatory form of cell death. In contrast, autophagy is a reversible process that can contribute both to tumor cell death and survival [4]. Many studies have subdivided programmed cell death (PCD) into the three categories: Apoptosis, Autophagy, and programmed Necrosis, based on criteria such as morphological alterations, initiating death signal, and the activation of caspases [5].

This in deed makes autophagy a unique point of interest as it is reversible and modifiable depending of the goal of therapy. Although apoptosis and autophagy are independent cell death pathways, but recently we have understood that inhibition of autophagy cases apoptosis, as in experiments only cells with a disrupted mitochondrial transmembrane potential were beyond the point of no return and inexorably died even under optimal culture conditions. Generally autophagy blocks the induction of apoptosis, and apoptosis-associated caspase activation shuts off the autophagic process. However, in special cases, autophagy or autophagy-relevant proteins may help to induce apoptosis or necrosis, and autophagy has been shown to degrade the cytoplasm excessively, leading to 'autophagic cell death'. The dialogue between autophagy and cell death pathways influences the normal clearance of dying cells, as well as immune recognition of dead cell antigens. Therefore, the disruption of the relationship between autophagy and apoptosis has important pathophysiological consequences [6].

The subject of interest and for that matter debate remains on what special circumstances or for that matter, genetic drivers for a tumor predisposes it to either induction of autophagy as proapoptotic or inhibition of autophagy as the tumor proapoptotic trigger. The debate is an essential one, as in clinical practice

prescribing medications that trigger apoptosis directly are not available, as such being able to promote apoptosis through an indirect mechanism (either induction of inhibition of apoptosis) becomes very relevant and critical.

There seem to also exist another reverse correlation between autophagy and apoptosis, as anything promoting apoptosis induces autophagy. Therefore it seems that there is a feedback loop that prevents the continuation of apoptosis by a reverse induction of autophagy. Many stimuli that ultimately cause cell death also trigger autophagy. In such cases, autophagy usually manifests well before apoptosis dismantles the cell. Autophagy induction is exacerbated if apoptosis is suppressed [7]. In addition, it seems that apoptotic caspase activation degrades essential autophagy proteins, shutting down the autophagic process and converting pro-autophagic proteins into pro-apoptotic ones (Figure 1).

The available evidence indicates that the cytosolic pool of p53 represses autophagy. This is an important information as common thought is that most tumors are initiated when there is lack of TP53 expression, this in deed challenges the common thought that reduced autophagy is involved in carcinogenesis, rather it appears that there is evidence quite opposite this [8]. Does this matter in prevention of cancer? It can as then treatments that inhibit autophagy can be considered for prevention of cancer. (However, it all depends when we are preventing cancer, as the prevention can be considered before or after a mutation has occurred). It appears that induction of autophagy is mostly protective before the mutation has occurred.

There are also data suggesting that MYC activation also elicits an unfolded protein response (UPR), which in turn activates cytoprotective autophagy via the endoplasmic reticulum stress kinase protein kinase RNA-like ER kinase (PERK) to favor cell survival. This is an important step in carcinogenesis, again dependent on autophagy [9].

That said, There is accumulating evidence that ageing is associated with a progressive dysfunction of autophagy, a condition that may favor the accumulation of dysfunctional mitochondria, nuclear genome instability, stem cell attrition, enhanced loss of post-mitotic cells, immunosenescence, unwarranted inflammatory reactions and, in the end, the reduction of hormetic responses that is required for the adaptation to stressful conditions. Moreover, autophagy is induced by, and required for, the positive influences of longevity-extending regimens, including caloric restriction, inhibition of insulin and insulin-like growth factor signaling, sirtuin 1 activation or long-term treatments with rapamycin, resveratrol or spermidine. Autophagy can also attenuate cell death by selectively reducing the abundance of pro-apoptotic proteins in the cytosol [10].

All of these information points into protective nature of autophagy against ageing. (and related diseases

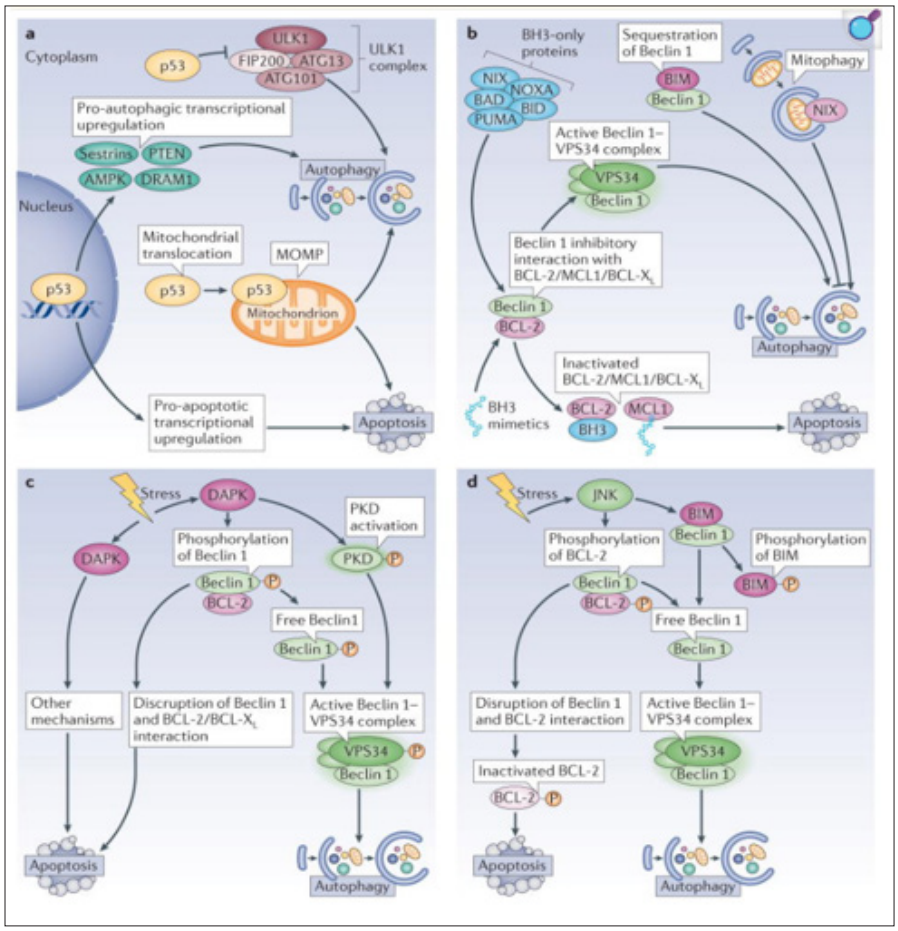

Figure 1: Signals that Induce Both Apoptosis and Autophagy

The genes involved in apoptosis control, including BCL-2 complex are also modulated by autophagy. It was not until this year, in 2025; that we realized the significance of BCL-2 molecule in modulating the relationship between autophagy and apoptosis. The inhibition of autophagy by Bcl-2 primarily occurs via direct binding to the BH3 domain of Beclin 1, a key autophagy regulator [11].

This dual regulatory function positions Bcl-2 as a key integrator of cellular stress signals. *Under nutrient deprivation*, AMPK activation phosphorylates Bcl-2, leading to its dissociation from Beclin 1, facilitating autophagy [12, 13].

(The nutrients of interest are mainly branched amino acids (Leucine, Isoleucine and Valine), methionine, arginine and glutamine).

Conversely, under nutrient-rich conditions, mTOR activation stabilizes the Bcl-2-Beclin 1 complex, thereby inhibiting autophagy and promoting cell survival. Furthermore, oxidative stress-induced phosphorylation of Bcl-2 determines whether the balance shifts toward autophagy or apoptosis. Targeting Bcl-2 to restore the balance between autophagy and apoptosis holds therapeutic potential for mitigating disease progression, enhancing cell survival and improving treatment response in these conditions [14].

RAS-mutant cancers (e.g., pancreatic, colorectal) utilize autophagy to maintain mitochondrial metabolism and handle the high metabolic demand of rapid proliferation. This phenomenon is unique and quite opposite of many other known genetic mutation in cancer. For example, in other genetically driven types of tumors, inhibition of m -tor pathway by fasting and other methods (medications such as everolimus) reduces tumor growth. In RAS driven tumors this is opposite. Activation of m -tor pathway reduces the autophagy and increases tumor regression! Interestingly Everolimus has been studied in KRAS mutated tumors with failed results (unless combined with EGRR inhibitors, such as afatinib)

Key Aspects of RAS Mutation and Autophagy: Survival Mechanism: RAS-mutant cancers (e.g., pancreatic, colorectal) utilize autophagy to maintain mitochondrial metabolism and handle the high metabolic demand of rapid proliferation.

Unique Non-Canonical Pathway: Oncogenic RAS induces a specific, distinct form of non-canonical autophagy called RINCAA (RAS-induced non-canonical autophagy via ATG8ylation), which differs from standard starvation-induced autophagy.

Therapeutic Targeting: Combined inhibition of the RAS pathway and autophagy (e.g., targeting PI4KB, CK1α, or using autophagy inhibitors) shows superior effects in slowing tumor growth compared to targeting either pathway alone Ras inhibitors in other hand, induce autophagy and therefore combination of RAS inhibitors with autophagy inhibition is reasonable [15].

In this context we decided to treat a positive NRAS patient with agents that inhibit autophagy and BCL-2 at the same time, as well as inhibition of m-tor pathway which could be downstream of MAPK and RAS. This theory proved to be effective in this case as we discussed here to be feasible and with positive outcome.

Methods and Materials

A 55 year old male with no history of diagnosed cancer, but positive findings in liquid biopsy for NRAS mutation and a positive liquid biopsy by NETEST detecting a neuroendocrine tumor was selected for a new therapeutic approach. (Standard of care in this case would be watchful waiting). Patient had already undergone a left adrenalectomy for a possible adrenal lesion which was diagnosed as adrenal nodule. Serial liquid biopsies consistently had shown presence of NRAS mutation since his diagnosis of adrenal mass, in May of 2024.

Liquid biopsy was performed through Guardant 360 laboratories with identification of NRAS G12D gene as mutated allele frequency of 0.3 percent reported in October 2025. Patient was consented and started on a regimen of therapeutics to inhibit autophagy, including Plaquenil (hydroxychloroquine), 100 mg twice a day. Hydroxychlorquine is a potent autophagy inhibitor, in fact second after Bafilomycin. (Antimalaria agents have dual effect on autophagy as some inhibit and some induce autophagy, such as artemnisine, Mefloquine and malarone). Plaquenil had to be stopped after 4 weeks due to rash.

Patient received oral Huitlacoche/ Corm smut (with similarities to wortmannin) on daily basis, as well as a D2/Calcium channel blocker known for it's inhibition of autophagy (Flunarizine, generally used to prevent migraines), at 2.5 mg twice a day, and medications that induced autophagy were discontinued (Celebrex, metformin and aspirin). Patient also stopped doxycycline which is a known autophagy inducer, and started quercetin 1 gram oral daily for epigenetic regulation/ histone demethylation of autophagy (see discussion). Corn smut, enriched in Leucin; has a strong effect on autophagy, PI3KCA inhibition and BCL-2 inhibition. This compound has been studied extensively by myself and patented in combination of other compounds.

Flurozinine was selected as a D2/Ca++ channel blocker that promotes degradation of RAS. effectively. Moreover it appears that the increased influx of Ca++ in cellular cytoplasm increases the cancer signaling and vice versa for neurodegenerative disorders. As such Ca++ channel inhibitors could be considered as useful tools in cancer therapeutics. (with consideration that these agents have about 2 percent chance of causing neurodegenerative diseases/ parkinsonism).

We also combined Chlorpromazine with Flurazinine (known for it's Ca channel blocking effect), as both these medications inhibit D 2 signaling and synergize in inhibition of autophagy. (Chlorpromazine is a strong inhibitor of fusion of lysosomes and autophasomes.

Patient maintained a high protein intake to reduce the risk of low nutrient activation of autophagy. The diet consisted high intake of branched amino acids (leucine, isoleucine) and methionine, and arginine. (red meat), all of which inhibit autophagy. Patient also took Crestor 10 mg a day as well as Black seed extract at high dose daily. (inhibitors of autophagy and AMPK respectively). High dose Protonix as another inhibitor of autophagy was considered but not tried.

Liquid biopsy was repeated after about 3 months (exactly 83 days) which showed drastic reduction to 0.1 percent. (see figure 2). Interestingly DNMT3A mutations were also detected with similar pattern as the NRAS G12D. This is not unexpected as the correlation between DNMT3A and NRAS has been documented in other types of cancer, such as leukemia. (through c Myc) Quercertin as such seemed to be a perfect therapy to inhibit such communication by inhibition of C Myc. FAAP 100 mutation however was observed as a new finding, which could be related to DNA damage repair insufficiency and poor prognosis. Such variation was deemed insignificant variant by the lab, but would require monitoring.

Patient had shown no side effects form the treatments provided, except for mild rash in his hands due to hydroxychloroquine.

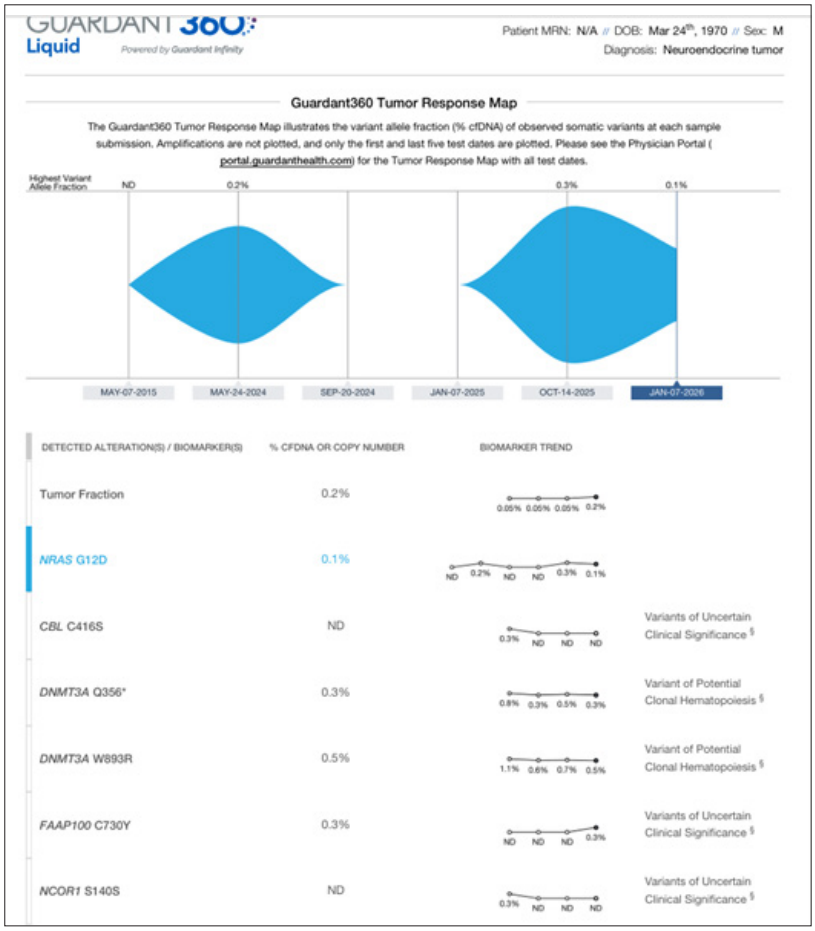

Figure 2: Liquid Biopsy Pre and Post Therapeutic Approach

Discussion

The literature is ambiguous when it comes to the role of autophagy in carcinogenesis and tumor progression. Many studies show a positive correlation between autophagy and tumor regression while others show completely opposite effect. In order to understand the data we need to look at the type of tumor or for that matter to be more specific the genes that are driving tumor growth. For some genes the autophagy seems to have a positive effect on inducing apoptosis versus on others specifically RAS mutated genes, and tumors driven by those alterations, autophagy seem to have completely different role. It actually helps the tumor cells to grow faster. Also when it comes to the stage of disease this correlation is important, as in stages of mutogenesis prior to mutation (gene amplifications and growth factor inductions), when there is no actual mutation yet detected, autophagy seem to be playing a healthy role in prevention of cancer, when after the mutated phase of carcinogenesis, this roles reverses. This phenomenon is very much like the effects of epigenetics in prevention of cancer, and it well could be related to epigenetic switches such as phosphorylation and ubiquitination of specific autophagy related genes. (Autophagy-related (ATG) genes are a conserved set of approximately 20 genes that regulate the formation of autophagosomes for cellular degradation and recycling). Most important of all in these genes are m-tor and Pi3K CA [16,17].

Genes Involved in Autophagy

Initiation: ULK1, ULK2, ATG13, RB1CC1 (FIP200).
Nucleation: BECN1 (Beclin 1), ATG14L, PIK3C3 (VPS34), PIK3R4 (VPS15).
Elongation (ATG12 Conjugation): ATG12, ATG5, ATG16L1.
Elongation (LC3 Conjugation): MAP1LC3 (LC3A/B/C), GABARAP family, ATG4, ATG7, ATG3.
Trafficking: ATG9A, ATG9B, ATG2A, ATG2B.

Importantly these genes are also under the influence of epigenetics, specially Key regulated genes include MAP1LC3B, ATG5, BECN1, and ATG7, controlled by enzymes like EHMT2/G9a, EZH2

This interaction has been well studied in cancer and neurodegenerative disorders, and autophagy known as a novel evolutionary phenomenon [18]. It has been found JMJD3, a histone demethylase reducing di- and tri-methylation of H3K27, regulated the expression of several key autophagy genes via demethylation of H3K27me3 at the gene promoters [19].

In our case study, we used a combination of products which are off label or natural including Plaquenil, (for strong inhiation of autophagy), quercetin (for strong demethylation of DNMT, and histone, as well as HDAC), We choose quercetin as all other standard HDAC inhibitors, induce autophagy by inhibiting m -tor. Quercetin in other hand inhibits Pi3K pathway and as such inhibits m -tor, but at the same time it reduces excessive autophagy. It also inhibits BCL-2 [20]. Other natural compounds used included Black seed extract. Black seed extract has strong BCL-2 inhibitory effect. (cotton seed extract, Withametelin/ datura Metel and Luteolin also manifest such properties). Such compounds strongly inhibit Ras protein.

We also used Flunarizine as this medicine is also a dual inhibitor/inducer of autophagy and specifically induces degradation of RAS molecule, and tested in different cell lines including GBM and TNBC [21].

Despite the importance of Ras in cancer, there is no drug yet that specifically targets Ras proteins. The most common "Ras inhibitors" actually target farnesyltransferases (farnesyltransferase inhibitors, FTIs) to reduce general membrane affinity of Ras proteins. However, FTIs can inactivate other prenylated proteins (≥2% of all proteins are prenylated) or cause some Ras proteins to instead undergo geranylgeranylation, thus retaining similar membrane affinity. These and other factors greatly reduce FTI effects on treating Ras-driven cancers. As such finding alternative drugs to treat RAS positive tumors are critical [22]. Chemotherapies as discussed earlier increase the tumor resistance and inhibit apoptosis by induction of autophagy/mitophagy.

Mitophagy is a prominent form of autophagy, Accumulating studies are suggesting that mitophagy may serve as a suppressor of cell death and promote cancer progression under cytotoxic stresses through effectively clearing damaged/detrimental mitochondria and thus helping the cancer cells to adjust the microenvironment of limited oxygen or nutrient and develop drug resistance. Mitophagy can enhance stem cells and Notch-1 pathway as well as RAS driven transformation. Combination of off label medicine that can inhibit autophagy (such as wortmannin) simultaneously with compounds that defragment and destroy mitochondria (such as magnolol or flurozinine) has been a great strategy to treat different cell lines, such as GBM. Wortmannin is able to efficiently block mitophagy through inhibition of LIR-mediated recruitment of primary mitophagy receptors, including OPTN and NDP52, thus to disrupt the second positive feedforward amplification loop of mitophagy and thus block mitophagy at the early stage. These findings led our study to examine the effects of Wortmanin in combination with Flurozinine.

Conclusion

Autophagy and apoptosis pathways are crosslinked with significant implication in both cancer prevention and treatment. Inhibition of autophagy pathways in certain types of cancer driven by RAS mutated genes can be considered a viable option to be explored in larger trials.

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