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oTargeting autophagy in *BRAF* mutant tumors

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Summary

Recent studies have highlighted the opportunity to treat cancer by inhibiting autophagy but have also raised important caveats with this idea. A paper in this issue of *Cancer Discovery* adds to accumulating evidence suggesting that we should focus our efforts (at least initially) on specific tumors where we are most likely to see beneficial effects.

Macroautophagy (which we will refer to as autophagy) is the process by which cellular material is delivered to lysosomes via vesicles called autophagosomes. Autophagy is widely viewed as being important in cancer and, in the last year, several clinical trials reported deliberate attempts to inhibit autophagy along with various other anti-cancer drugs in multiple tumor types. The key word in the previous sentence is “deliberate” – we also know that many anti-cancer agents inadvertently alter autophagy (in some cases stimulating and in others inhibiting the process). This means that even during standard therapy, autophagy is likely being manipulated in cancer patients whether we want to do so or not. Is such autophagy manipulation a good thing or a bad thing to be doing? The big issue is that autophagy has multiple, often competing, effects on tumor cell behavior (1) and, autophagy inhibition could therefore sometimes be good and sometimes bad from the perspective of a cancer patient (2). This creates a conundrum– how should we try to manipulate autophagy in cancer patients and should we be doing the same in everyone?

A paper (3) in this edition of *Cancer Discovery* from the laboratories of Janice Mehnert and Eileen White adds to accumulating evidence that *BRAF*mutant tumors are good candidates for deliberate autophagy inhibition. The authors use a genetically engineered mouse model (GEMM) of *BRaf* mutant, *Pten*-null melanoma to show that autophagy inhibition causes tumor growth inhibition leading to extended survival of the mice. This work follows several studies concluding that autophagy inhibition can have anti-tumor effects especially on tumors driven by RAS pathway mutations. For example, an elegant paper also from Dr. White’s group showed a profound anti-tumor effect on *k-Ras*-driven murine lung tumors when autophagy was acutely inhibited by whole body knockout of the *Atg7* gene (4). Similar anti-tumor effects and extension of life span have been seen in *k-Ras*-driven pancreas tumors (5, 6) and *BRaf*-driven murine lung cancers (7) when autophagy is blocked in tumor cells by knockout of essential autophagy genes such as *Atg5* or *Atg7*. Genetic and pharmacological

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inhibition of autophagy in human melanoma cells and xenografts with the *BRAF*^{V600E} mutation can also overcome resistance to RAF inhibitors (8). Similarly, brain tumor cells with the same *BRAF* mutation are killed by autophagy inhibition even in the absence of extra stressors and a patient with a *BRAF* mutant brain tumor was successfully treated with the autophagy inhibitor chloroquine to overcome acquired resistance to the RAF inhibitor vemurafenib (9). Importantly, this new study (3) shows that deletion of *Atg7* improves the effectiveness of the *BRAF*^{V600E} inhibitor dabrafenib in a melanoma GEMM. Thus a pattern seems to be emerging whereby RAS pathway-driven and especially *BRAF* mutant tumors have increased dependence on autophagy allowing effective treatment with autophagy inhibition and improving RAF inhibitor therapy effectiveness.

However, while it is clear that disruption of autophagy in these tumor models usually has a profound effect on metabolism and causes accumulation of damaged mitochondria, there are also potentially important differences in the responses of tumors in the various models. Sometimes autophagy-dependent tumor cells die when autophagy is inhibited even in the absence of added stress (9), but in pancreas tumors the effect of autophagy inhibition is primarily on cell growth, not survival (5). Xie et.al. (3) add another phenotype because, in this study, autophagy inhibition caused melanoma cell senescence, while also potentiating dabrafenib-induced senescence. There's also some confusion regarding the role of RAS, which is the immediate upstream activator of RAF. In most studies tumors with mutant RAS respond to autophagy inhibition, however this can be context dependent too with some tumor cells displaying growth inhibition when autophagy is blocked with chloroquine and others showing the opposite effect even when they have the same oncogenic *RAS* genes (10). Some of the variable effects may be due to when autophagy is inhibited. In most of the GEMMs, autophagy was inhibited simultaneously with activation of the oncogenic signal so that the tumor developed with no ability to activate autophagy. This may be different from those situations where autophagy is inhibited in a fully formed tumor as would be the case when treating people.

Most important, it has been questioned whether any benefits of autophagy inhibition could be outweighed by toxicity from blocking this process. Most mouse studies, including the latest one (3), inhibited autophagy only in the tumor cells. In humans we can't do that. If we inhibit autophagy in people, we will do so with drugs like chloroquine, which will affect both tumor cells and the rest of the body. Will that cause problems? The clinical studies with chloroquine and hydroxychloroquine have shown few signs of toxicity. For example, one patient whose brain tumor is being treated by autophagy inhibition with chloroquine (9) has been treated with a combination of chloroquine and vemurafenib for over two years with no signs of toxicity from the autophagy inhibitor. However, one area of unusually high agreement in the autophagy field is that chloroquine is a poor autophagy inhibitor especially *in vivo*. For instance, it has other effects in addition to blocking autophagy. In addition, chloroquine levels that are achievable in tumors are variable and, even in the best cases, probably are only just enough to inhibit autophagy. So, the trivial explanation for why this drug is well tolerated is that it is doing a poor job of inhibiting autophagy. Karsli-Uzunbas et.al. (4) modeled a "perfect" autophagy inhibitor by studying the effect of an acute whole body knockout of an essential autophagy gene (*Atg7*) in adult mice with cancer. The

encouraging result was that the tumors went away, however, a cup-half-empty view of this study is that the mice died anyway because autophagy inhibition caused neurodegeneration after a few weeks. Obviously, a cancer treatment that caused tumors to go away but led to death by neurodegeneration shortly afterwards is not much use. Thus a critical question for the field is whether a useful anti tumor response will require that we inhibit autophagy so effectively that the beneficial effects would be outweighed by toxicity on normal tissues especially the brain. Xie et.al. (3) addressed this question and found that chloroquine treatment was, like *Atg7* knockout, an effective way to suppress the same $BRaf^{V600E}$ driven tumors. This is important because it suggests that if you treat the right sort of tumors (i.e. those that really depend on autophagy), even a drug that we all agree is a pretty lousy autophagy inhibitor can inhibit autophagy enough to get the anti-tumor responses we want without overt toxicity. In cancer medicine this sort of thing often comes up– e.g. global and irreversible knockout of topoisomerases would likely be very toxic in a mouse, but this doesn't mean we can't use topoisomerase inhibitors in the clinic. The key is to inhibit the target (in this case the process of autophagy) incompletely– usually not a problem with drugs, which unlike knockout of genes that are essential for a given process, never work with 100% efficiency– but enough to get tumor-specific effects. In this view, autophagy inhibition is simply another case where we have to balance benefits and costs and come up with ways to identify patients who will and won't benefit from the treatment.

There are still many important questions we need to answer. Why does *RAF* mutation make tumor cells so reliant on autophagy? Why do some tumor cells die and others don't upon autophagy inhibition, why do some become senescent? Why are there differences amongst *RAS* mutant tumors? Are there similarities amongst autophagy-dependent tumors that will allow us to identify them all even if they don't have mutant *RAF*? Is the autophagy that is often induced by other anti-cancer drugs (as opposed to the basal autophagy that is going on all the time in mammalian cells) especially important for determining whether a tumor cell will live or die? Can we make better GEMMs that will allow us to model reversible and different extents of inhibition of autophagy and thus determine when and how much autophagy inhibition is needed to maximize beneficial effects while minimizing toxicity in cancer treatment?

The information we already have, however, suggests that to effectively treat cancer by autophagy inhibition we'll need at least two things: 1) a drug to inhibit autophagy preferably with better pharmacological properties and more specificity than chloroquine, and, 2) a way to identify people whose tumor is autophagy-dependent. The accumulating evidence in melanoma, lung and brain tumors with human cell lines, genetically engineered mice, human tumor xenografts and even the first patients suggest that *BRAF* mutation may be a good place to start looking for autophagy-dependent tumors to test these ideas.

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