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The formation of autophagosomes during lysosomal defect: a new source of cytotoxicity

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Macroautophagy/autophagy comprises autophagosome synthesis and lysosomal degradation. It is well known that lysosomal defects cause toxicity to cells. However, it has not been investigated previously if cytotoxicity is conferred by autophagosome formation during lysosomal defect. Recently, we found that the formation of autophagosomes in such conditions also causes cytotoxicity, in addition to lysosomal defect insults. We revealed that a partial reduction in autophagosome synthesis was beneficial for cell survival in cells bearing the autophagosome formation-based toxicity. Our study suggests that production/accumulation of autophagosomes during lysosomal defect directly induces cellular toxicity, and this process may be implicated in the pathological conditions where lysosomes are defective.

Keywords: autophagosome, autophagy, cytotoxicity, MTOR, STX17

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The autophagy process includes autophagosome biogenesis, autophagosome-lysosome fusion and cargo degradation. In certain stress conditions, increased autophagosome synthesis may be associated with decreased lysosomal activity, which results in reduced clearance of autophagosomes by the rate-limiting lysosomal activity. It is well known that lysosomal defect causes cellular toxicity, since all lysosome-related pathways, including autophagy, are dysfunctional in such conditions. However, it is unknown if autophagosome formation itself during lysosomal failure also causes toxicity. One can expect that the synthesis of autophagosomes in such conditions may yield no survival benefit, since they cannot be cleared and recycled when lysosomes are defective. We have now extensively demonstrated that the formation of autophagosomes that subsequently fail to fuse with lysosomes directly induces cytotoxicity.

We initially observed that increased autophagosome accumulation during lysosomal defect occurs with cell viability loss or cell death. Therefore, we asked if autophagosome formation *per se* causes cytotoxicity under these conditions, in addition to the lysosomal-defect toxicity. To establish a model for autophagosome formation coupled with lysosomal defect, we first simultaneously employed siRNAs for *MTOR*, encoding an autophagosome synthesis repressor, and *STX17*, encoding a SNARE protein responsible for autophagosome-lysosome fusion. Treatment with *MTOR* and *STX17* siRNA therefore results in autophagosome formation induction, and a blockade to autophagosome-lysosome fusion, respectively. Interestingly, we found that dual knockdown of MTOR/STX17 synergistically induces significant toxicity at a much higher degree than that of either of them alone. These data suggest that autophagosome formation during lysosomal defect can induce toxicity independent of

lysosomal defect insults. We replicated MTOR and STX17 dual knockdown toxicity by substituting STX17 for the alternative target VPS33A, a member of the HOPS complex that is also required for autophagosome-lysosome fusion. This approach was to ensure that the viability loss from our dual knockdown treatments was attributed to increased autophagosome formation coupled with inhibition of autophagosome-lysosome fusion. Consistently, we observed that MTOR and VPS33A dual knockdown also synergistically cause cell viability loss.

Because MTOR ablation may affect additional cellular pathways other than autophagosome synthesis, we also attempted to target an MTOR-independent pathway to exclude any potential cytotoxicity effects from other pathways in our MTOR and STX17 or MTOR and VPS33A dual knockdown assays. Studies have shown that reductions in free inositol lead to enhanced autophagosome synthesis, independently of MTOR pathways. As such, using siRNAs we targeted IMPA1 (inositol [myo]-1[or 4]-monophosphatase 1), an MTOR-independent autophagy regulator, along with SNAP29 (which is important for autophagosome-lysosome fusion) to mimic the role of MTOR and STX17 for cytotoxicity induction. Consistently, while knockdown of either IMPA1 or SNAP29 alone leads to a modest reduction in cell viability, IMPA1 and SNAP29 dual knockdown dramatically enhance cytotoxicity. Importantly, we showed that the cytotoxicity from MTOR and STX17 or IMPA1 and SNAP29 dual knockdown is alleviated when autophagosome formation is specifically inhibited by *ATG* gene knockdown. Chemical treatments were also used to consolidate the data from genetic approaches. Treatment with the dual PI3K and MTOR inhibitor PI-103 induces both autophagosome accumulation and cell death. Interestingly, whereas the lysosome inhibitor bafilomycin A_1 exacerbates the cell death caused by PI-103, blocking

autophagosome formation with 3-methyladenine provides significant protection from PI-103 lethality. Together, these data indicate that the formation of autophagosomes that subsequently fail to fuse with lysosomes (during lysosomal defect) confers cytotoxicity.

We found the toxicity to occur independently of the 2 major cell death routes, apoptosis and necroptosis, because chemical and genetic inhibition of either pathway fails to afford any cytoprotective effects. We sought to reveal the sources of toxicity, and found that an increase in reactive oxygen species (ROS) production and intracellular energy deficit at least in part contribute to the toxicity. As lysosome defects mean that autophagosomes and their contents cannot be degraded and recycled, continued autophagosome synthesis only serves to deplete cellular energy and nutrition levels, as well as generate increased ROS, thereby resulting in toxicity. Because the continued formation of autophagosomes during lysosomal failure (or autophagosome accumulation) confers the cytotoxicity, we designated it as autophagosome accumulation toxicity.

Autophagosome accumulation toxicity could have implications in many disease conditions. We have shown that partially reducing the formation of autophagosomes alleviates the toxicity from mutant HTT (huntingtin) and SNCA (synuclein, alpha). Strategies of inducing autophagosome accumulation toxicity could represent a novel approach in future tumor treatments. A number of pathological conditions are associated with lysosomal defects, such as neurodegenerative disorders featuring toxic protein aggregates, and lysosomal-storage diseases. Therefore, autophagosome accumulation toxicity may be of particular interest and relevance to

studies into these diseases. Currently, inducing autophagy via stimulating autophagosome formation has been adopted as a frequently used strategy to attempt to treat these diseases. Our study argues that such a strategy of inducing autophagosome formation during periods of lysosomal defect will not be beneficial, but in fact detrimental to cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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