autophagy by Bafilomycin A1 treatment sensitized HCT116 colon cancer cells to Nutlin-induced apoptosis. These studies demonstrated that autophagy can promote survival in response to MDM2 antagonists like Nutlin by promoting degradation of FOXO3a and thus preventing PUMA expression. We hypothesize this mechanism could also promote survival in response to other agents that induce apoptosis in a PUMA-dependent manner.

Others have examined the effect of autophagy in response to radiation and chemotherapy and the involvement of p53 in this response. For example, Seiwert *et al.*[61] examined autophagy in response to DNA double strand breaks (DSBs) induced by ionizing radiation or the bacterial cytolethal distending toxin (CDT) in HCT116 colon cancer cells. They found DSBs induced autophagy dependent on ATM kinase and p53. Importantly, they found the autophagy inhibitor chloroquine sensitized cells to killing by CDT, supporting the idea that p53-dependent autophagy protects cells from agents like ionizing radiation and CDT that induce DSBs. Related to this are studies from the Gerwitz group. In their study they examined radiation-induced autophagy in breast, colon, and lung cancer cell lines that vary in p53 status or had p53 deleted by shRNA. They found that radiation could induce autophagy regardless of p53 status. Interestingly, however, autophagy inhibition sensitized p53 wild-type cells to radiation-induced killing but not cells that lacked wild-type p53[62]. These findings raised the possibility that the cytoprotective (survival) effect of autophagy in irradiated cells is dependent on wild-type p53. Alternatively, the results could mean autophagy inhibition sensitizes cells to radiation in a p53-dependent manner. Studies by Zeng *et al.*[63] examined the relationship between autophagy and apoptosis in mismatch repair (MMR) proficient and deficient colon cancer cells treated with the chemotherapy agent 6-thioguanine (6-TG). The authors found that 6-TG treatment induced autophagy dependent on MMR activity and dependent on p53. Knockdown of the critical autophagy regulator ATG5 or pharmacologic inhibition of autophagy sensitized 6-TG treated cells to apoptosis. While the mechanism of how autophagy protects cells from 6-TG was not determined, the results nonetheless indicated p53-mediated autophagy can protect cancer cells from killing by the therapy agent 6-TG[63].

Finally, another possible mechanism by which autophagy could protect cells from p53-induced apoptosis comes from studies of p53 in replication stress. Wild-type p53 is activated in response to replication stress, and recent studies have shown that p53 promotes replication fork processivity that may contribute to its tumor suppressor function[64]. In unpublished studies, we have gained evidence that p53 induced by the replication stressor hydroxyurea (HU) promotes autophagy, and that bafilomycin A1 co-treatment sensitizes HU-treated cells to apoptosis. Vanzo *et al.*[65] recently reported that autophagy can help maintain replication forks in response to replication stressors by maintaining nucleotide levels. Based on this, we speculate autophagy may also protect cells from p53-induced killing in response to replication stresses by maintaining nucleotide levels.

Autophagy can contribute to p53-mediated apoptosis

While the studies described above indicate autophagy can protect cells from p53-mediated death/apoptosis in response to radiation and certain therapy agents, other studies suggest the opposite. One example is the study by Borthakur *et al.*[66] in which they examined autophagy and apoptosis in Nutlin-treated acute myelocytic leukemia (AML) cells. They found Nutlin induces autophagy in AML cells in a manner that appears to involve p53 activation of AMPK and subsequent inhibition of mTORC1. Notably, in their study, autophagy inhibition by Bafilomycin A1 reduced apoptosis in Nutlin-treated AML cells, supporting the idea that autophagy induction contributed to apoptosis[66]. Another example is the study by Kenzelmann Broz *et al.*[23], described above, in which ChIP-seq and RNAseq were used to identify autophagy genes regulated by p53 in MEFs treated with the DNA damaging agent doxorubicin. In that study, it was found that p53 bound and activated expression of multiple ATG genes and promoted autophagy in response to doxorubicin treatment. Inhibition of autophagy by ATG5 knockout reduced

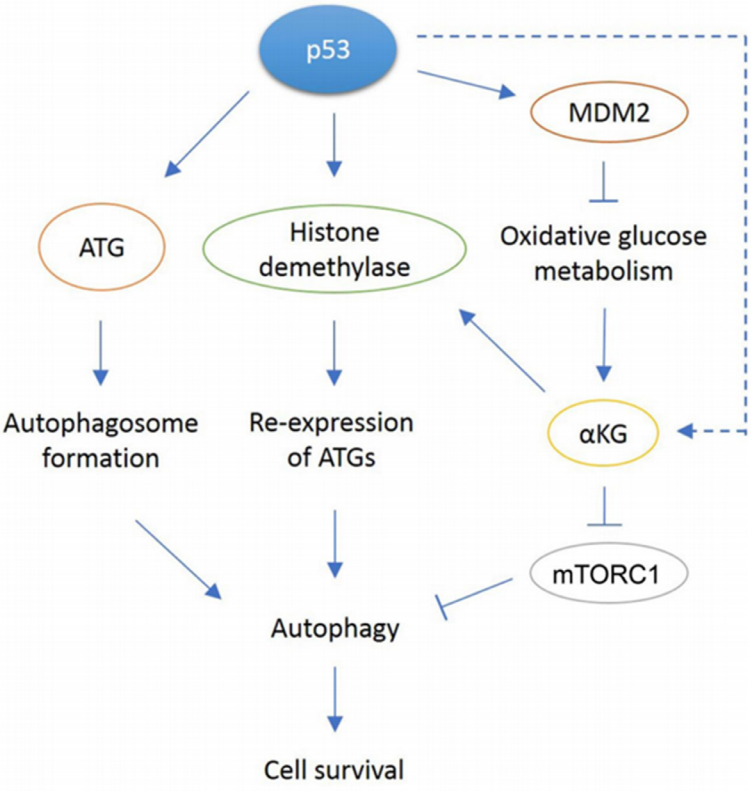

Figure 1. In response to cellular stress, p53 can promote autophagy through various mechanisms. p53 can directly bind the conserved binding site in ATG gene promoters and transcribe proteins required for autophagosome formation. p53 can also induce transcription of JMJD2B demethylase that removes methylation on histone H3, allowing re-expression of previously repressed ATGs. Another proposed mechanism is through p53-mediated oxidative metabolism. Through activation of multiple target genes, p53 can shift metabolism away from glycolysis to favor oxidative metabolism instead. The reduction in glycolysis has been observed only in MDM2 amplified tumor cells. A resulting metabolite, αKG is a cofactor for JMJD2B, so it may be possible to play a role in histone modification that leads to re-expression of ATGs. Our paper showed αKG levels decreased in MDM2-amplified cells treated with Nutlin but increased in response to Nutlin in MDM2 non-amplified cells through an unknown mechanim (dotted arrow). Also, αKG may be involved in mTORC inhibition as observed in C elegans

p53-dependent apoptosis in response to doxorubicin, supporting the idea that p53-mediated autophagy contributes to doxorubicin induced killing[23]. Yet another example is the study by Gao *et al*.[67] In their study, U2OS osteosarcoma cells were treated with camptothecin or etoposide. The authors found that p53 induced autophagy in response to both treatments, and that inhibiting autophagy rescued the cells from camptothecin-induced killing.

CONCLUSION

There are several reports that demonstrate autophagy can protect cells from p53-mediated apoptosis and cancer cell killing in response to radiation, chemotherapy, and small molecule MDM2 antagonists. These findings would support the potential for combining autophagy inhibitors with therapy agents that stabilize and/or activate p53 to improve cancer cell responses. However, there is also evidence that autophagy can contribute to p53-mediated killing in cells exposed to MDM2 antagonists and certain therapeutic drugs. Thus, the impact of autophagy on p53-mediatd apoptosis and cancer cell killing in response to radiation and therapeutic drug treatment is likely cell-type and context dependent. A better understanding of how autophagy regulates cell fate in response to activated p53 will be required for future consideration of autophagy inhibitor usage in cancer patients.

P53 can promote autophagy through multiple mechanisms. These mechanisms include direct transcriptional activation of ATG genes by p53, and indirect regulation of these genes by p53 through alterations in glycolysis, histone methylation, and α-KG levels [Figure 1]. While Figure 1 depicts autophagy as a general survival mechanism, it is important to note that autophagy can also have a tumor suppressive role that appears dependent, at least in part, on cancer stage[68]. In response to cancer therapy agents, tumor cells can manipulate autophagy to promote tumor survival[69]. While more work is needed, inhibition of autophagy may be considered as a potential treatment adjuvant in patients who display chemo and/or therapy resistance.

DECLARATIONS

Authors' contributions

Conceived the manuscript outline; wrote and edited the first draft; made the figure: Shim D
Conceived the manuscript; edited the draft and final manuscript: Duan L, Maki CG

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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