Autophagy inhibition enhances therapy-induced apoptosis in a *Myc*-induced model of lymphoma

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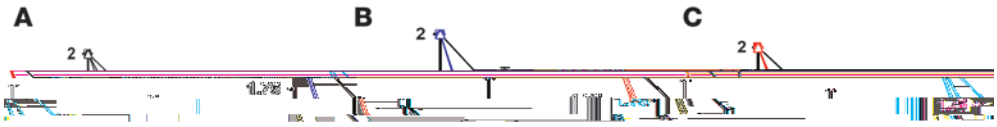
ment with MNNG and CQ resulted in 9-fold and 17-fold increases in punctate LC3 fluorescence compared with DMSO control at 24- and 48-hour time points (Figure 7D). To further compare the effect of genetic autophagy inhibition with that of CQ following cytotoxic therapy, the effect of MNNG treatment with and with-



genesis may be related to the reported function of autophagy in eliminating damaged or excess organelles (35, 36). Yeast defective in *1,* which encodes a mitochondrial protein required for effective targeting of mitochondria for autophagic degradation (37), are hypersensitive to certain types of oxidant injury (38). Therefore, chronic suppression of autophagy over a long period of time would result in the accumulation of cellular oxidants that damage DNA, increasing the likelihood of cellular transformation. The role of autophagy in suppressing the accumulation of oxidative damage to cells may account for autophagy's role in suppressing tumorigenesis while serving a tumor-protective effect against various types of cellular stress encountered by established tumors.

The present studies were undertaken because of numerous reports of autophagy observed in established cancer cell lines following anticancer therapy (1–3, 39). The data presented above demonstrate the induction of autophagy in tumor cells in vivo in response to the activation of p53, a gene commonly induced by a number of antineoplastic therapies. It has been suggested that the ability of radiation or chemotherapy to induce cell death in cancer cell lines that display resistance to apoptosis depends on type II programmed cell death executed by autophagy (40). In -induced lymphoma cells, knockdown of ATG5, an essential autophagy gene, did not impair p53-induced cell death, suggesting that the autophagy program that is activated with apoptosis does not contribute to cell death. Instead, autophagy is serving a survival function in this context since expression of shRNA directed against *A 5* in lymphoma cells augmented cell death following p53 activation. These data indicate that autophagy can be an adaptive response that allows cancer cells to survive an apoptotic stimulus that would otherwise lead to their demise.

The ability of tumor cells expressing *A 5* shRNA to grow suggests that once neoplastic proliferation is established, autophagy is not absolutely required for in vitro cell growth and survival. However, our data suggest that there is ongoing autophagy during */ 53E A* tumor growth, based on the accumulation of autophagic vesicles when their clearance by lysosomes is inhibited by CQ. When tumor cells are faced with cellular stress that induces apoptosis, autophagy serves to protect against cell death. Inhibition of autophagy in the setting of an apoptotic stress enhances apoptosis. Since autophagy contributes to cell survival in times of stress, it seems likely it can provide enhanced survival to tumor cells subjected to potentially catastrophic stress even when expressed in a haploinsufficient manner.

The tumors and tumor cells used in this study to investigate the role of autophagy following apoptotic stress were created by expressing wild-type human - in mouse bone marrow cells from the p53ER-knockin mouse. In addition to Burkitt lymphoma, in which a chromosomal translocation juxtaposes the - gene to 1 of 3 immunoglobulin genes, leading to its constitutive expression in B cells (41), dysregulation of - through gene amplification (42–44), - point mutations (45), or constitutive transcriptional and posttranslational activation (46, 47) has been implicated in the pathogenesis of multiple malignancies, including lung cancer, colon cancer, and breast cancer. The role of Myc as a transcriptional regulator of genes that affect glucose metabolism and its propensity to induce apoptosis (48) when expressed acutely in nontransformed cells raises the possibility that autophagy observed in these tumors may be a specific consequence of introducing a therapeutic stress on top of -induced cellular stress. Recently, however, autophagy was found to promote tumor survival in another tumor model driven by the oncogene *A* (49). Akt, when activated, also increases glycolytic metabolism and controls apoptosis. In these tumors, autophagy is activated and preferentially protects tumor cells in the centers of growing *A* -driven, apoptosis-deficient tumors, where nutrients and oxygen are limited. This suggests that oncogenes that activate the War-

burg effect, such as Akt, and drive the glycolytic phenotype frequently seen in a variety of tumors may predispose tumor cells to increase autophagy as a strategy for survival in the face of nutrient limitation. However, in / 53E A cells, the permeable nutrient methyl pyruvate did not rescue the enhanced p53-induced apoptosis observed in tumor cells in which autophagy was genetically silenced. Autophagy can promote cell survival not only by

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