Dear Editor,

DNA-damaging anti-cancer drugs cause cell death by apoptosis, but they also activate macroautophagy\(^1\) (hereafter just autophagy), a fundamental survival pathway under stress where cells enclose cytosol or organelles in double-membrane autophagosomes, then fuse them with lysosomes for recovery of metabolic precursors.\(^2\) This process depends upon autophagy-related (ATG) proteins.\(^2\) Activation of this survival pathway is unwanted in cancer therapy, because even a few surviving tumor cells can accumulate mutations, gain genetic diversity, and, potentially, resume proliferation.\(^3\)

We recently reported that expression of ATG5 was upregulated by treatment with low concentrations of etoposide.\(^4\) Few cells entered apoptosis, but almost all showed autophagy. Surprisingly, much of the induced ATG5 was found in the cell nucleus, binding to BIRC5/survivin and causing cell cycle arrest at G\(_2\)/M followed by mitotic catastrophe.\(^4,5\) We then asked whether just ectopic ATG5 expression, without etoposide, would lead to mitotic catastrophe.\(^4\) DNA damage, however, was not in evidence; neither ATM nor ATR phosphorylation was detected and foci of H2AX phosphorylation were absent.\(^4\) This has led us to ask: how can ATG5 induce the same kind of stress response as DNA-damaging drugs?

This report documents increased p53 expression after lentivirus-mediated, ectopic ATG5 expression (Figure 1a). Upregulated p21 demonstrated p53 transactivation of a target gene. Vector alone elicited only a slightly elevated p53. As expected, ATG5 expression also caused autophagy as measured by lipidated LC3 (LC3-II).\(^6\)

One wonders whether the observed p53 upregulation/ activation is necessary to initiate autophagy?\(^7\) Using p53 null Saos-2 cells made DOX inducible for p53 with a Tet-on construct, we showed that p53 was not required for autophagy (Figure 1b). Ectopically expressed ATG5 is shown as both 33-kDa monomer and 57-kDa conjugate with ATG12. Autophagy was apparent from LC3-II. An additional stimulus with nutrient starvation was followed within 1 h by further increased LC3-II, but without any requirement for p53 induction (Figure 1b).

As lentivirus-mediated gene transfer might have produced some local anomalies in DNA, we studied p53 expression/activation in an ATG5 knockout mouse embryo fibroblast (mEF) line that had been subsequently transfected with a Tet-off ATG5 expression system (clone M5-7).\(^7\) As these cells had been maintained since 2006 without DOX stimulation, a DNA damage response seems unlikely; however, we examined ATM phospho-Ser1981 as confirmation (Figure 1c). After DOX treatment, ATG5 expression in these cells was rapidly suppressed. Note: please ignore the unspecific band seen in mEF cells at 36 kDa. In mEF cells, ATG5 exists almost entirely as a conjugate with ATG12. Corresponding to the downregulation of ATG5 after DOX, both p53 expression and autophagy, monitored as LC3-II levels, declined sharply (Figure 1c). Also in Figure 1d, using the same Tet-off ATG5 expression system, these differences between ATG5-non-expressing and ATG5-expressing cells were apparent. Noteworthy is that, ATG5-expressing cells showed not only elevated p53 levels, but also increased p53 activation as evidenced by p53 Ser18 phosphorylation (Figure 1d). Interestingly, starvation also induced p53 in DOX-treated cells not expressing ATG5, not, however, LC3-II or autophagy.

Our findings thus indicate that increased ATG5 expression represents for the cell something like a stress response. In consequence, p53 upregulation and transactivation of p21 are followed by cell cycle arrest. Here p53 is not acting in its generally accepted role as a regulator of autophagy and apoptosis,\(^6\) but itself exhibits a secondary response to upregulation of ATG5 expression. Similar findings have been reported recently with ATG7 knockout mEF cells,\(^8\) showing that ATG7 also can impose a reciprocal regulation on p53. Surprisingly too, activation of the NF-\(\kappa\)B pathway, an important stress response, is blocked in cells lacking either ATG5 or ATG7.\(^9\) All these observations suggest a network of interactive responses available to the cell, initiated by different homeostatic imbalances, but integrated in the same overall program.

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**ATG5 can regulate p53 expression and activation**

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Conflict of Interest
The authors declare no conflict of interest.

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