Autophagy 8:2, 278–280; February 2012; © 2012 Landes Bioscience

MAP1S enhances autophagy to suppress tumorigenesis

Leyuan Liu,* Wallace L. McKeehan, Fen Wang and Rui Xie
Center for Cancer and Stem Cell Biology; Institute of Biosciences and Technology; Texas A&M Health Science Center; Houston, TX USA

Keywords: autophagy, C19ORF5, genome instability, hepatocarcinomas, LRPPRC, MAP1S, microtubules, mitochondria, p62, RASSF1A

Abbreviations: C19ORF5, chromosome 19 open reading frame 5; DEN, diethylnitrosamine; DNA DSB, DNA double-strand break; HCC, hepatocarcinomas; LC3, microtubule-associated protein 1 light chain 3; LRPPRC, leucine-rich PPR motif-containing protein; MAP1A, MAP1B and MAP1S, microtubule-associated protein family 1 A, B and small form; p62, sequestosome 1; RASSF1A, Ras association domain family 1 isoform A

Submitted: 11/12/11

Revised: 11/27/11

Accepted: 12/02/11

http://dx.doi.org/10.4161/auto.8.2.18939

*Correspondence to: Leyuan Liu;
Email: lliu@ibt.tamhsc.edu

Punctum to: Xie R, Wang F, McKeehan WL, Liu L. Autophagy Enhanced by Microtubule and Mitochondrion-associated MAP1S Suppresses Genome Instability and Hepatocarcinogenesis. Cancer Res 2011; 71:7537–46; PMID:22037873; http://dx.doi.org/10.1158/0008-5472.CAN-11-2170

Microtubule-associated protein 1 small form (MAP1S; originally named C19ORF5) was identified as serving as linkers to connect mitochondria with microtubules for trafficking, and to bridge the autophagy machinery with microtubules and mitochondria to affect autophagosomal biogenesis and degradation. We found that MAP1S levels become elevated immediately in response to diethylnitrosamine-induced or genome instability-driven metabolic stress in a murine model of hepatocarcinoma. Elevation of MAP1S enhances autophagy to remove p62-associated aggresomes and dysfunctional organelles that trigger DNA double-strand (DSB) breaks and genome instability. The early accumulation of an unstable genome prior to signs of tumorigenesis suggested that genome instability causes tumorigenesis. After tumorigenesis, tumor development then triggers the activation of autophagy to reduce genome instability in tumor foci. We concluded that an increase in MAP1S levels triggers autophagy in order to suppress genome instability so that both the incidence of diethylnitrosamine-induced hepatocarcinogenesis and malignant progression are suppressed. Thus, a link between MAP1S-enhanced autophagy and suppression of genomic instability and tumorigenesis has been established.

Autophagy marker LC3 was originally identified as an interactive partner of the microtubule-associated proteins MAP1A and MAP1B that distribute exclusively in neurons. Recently, we identified a ubiquitously-distributed microtubule-associated protein homolog, MAP1S. In addition to its interaction with LC3 as predicted, it interacts with tumor suppressor RASSF1A and the mitochondrion-associated protein LRPPRC (**Fig. 1A**) that also interacts with the mitophagy-related proteins PARKIN and PINK1, which triggered us to investigate the relation between autophagy and tumor suppression.

We created a *MAP1S* knockout mouse model in which both basal autophagy for clearance of abnormal mitochondria and nutritive stress-induced autophagy for nutrient recycling are impaired. We challenged half-month-old littermates of wild-type (+/+) and MAP1S-depleted mice (−/−) with a single dose of diethylnitrosamine (DEN) to induce hepatocarcinomas (HCC). The MAP1S-depleted mice had significantly higher tumor incidence, average number of surface tumors per mouse and ratio of liver weight to body weight (LW/BW) than the wild type at the same ages. Therefore, *MAP1S* is characterized as a tumor suppression gene.

To understand how MAP1S suppresses DEN-induced HCC, we traced the immediate responses of hepatocytes to DEN exposure for two weeks. Levels of MAP1S in hepatocytes were undetectable by immunoblotting but dramatically elevated immediately upon DEN exposure and then reduced to undetectable levels 2 d after exposure. Acute elevation of MAP1S leads to activation of both autophagy initiation and autophagosomal degradation, but with different degrees so that a large amount of LC3-II-associated autophagosomes and p62-associated ubiquitin-positive Mallory-Denk bodies accumulate. Depletion of MAP1S results in autophagy inefficiency so that much higher levels of p62 remain in hepatocytes even at two weeks after DEN exposure.

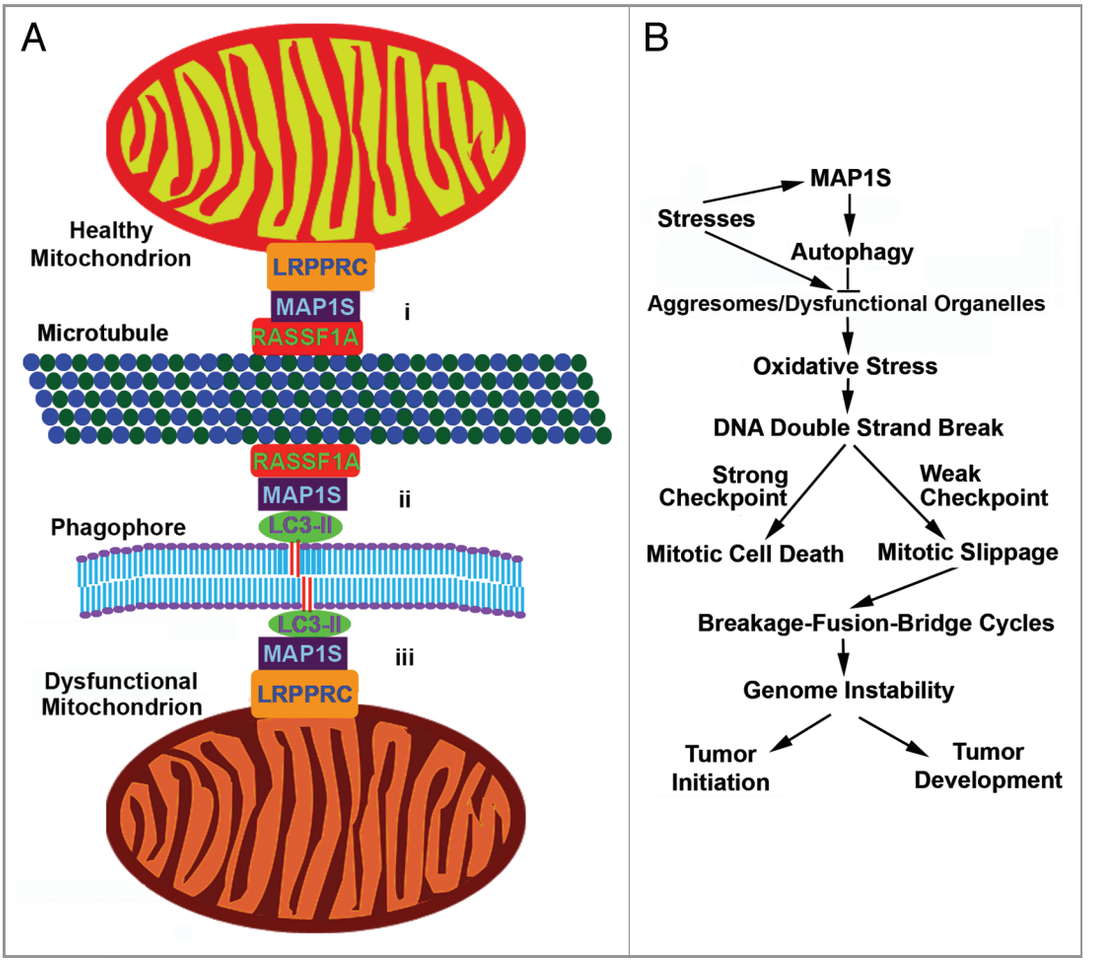

Figure 1. MAP1S promotes autophagy and suppresses tumorigenesis. (A) A model showing the function of MAP1S. (i) MAP1S bridges healthy mitochondrion to microtubules for trafficking, with the assistance of RASSF1A; (ii) MAP1S interacts with external LC3-II and bridges autophagosomes to microtubules for trafficking with the assistance of RASSF1A; and (iii) MAP1S binds with mitochondrion-associated LRPPRC and docks a dysfunctional mitochondrion into an autophagosome through the interaction with internal LC3-II. (B) MAP1S activates autophagy to suppress tumorigenesis. Stress resulting from carcinogen- or genome instability-driven metabolic stress causes accumulation of aggresomes or dysfunctional organelles such as mitochondria, and also enhances MAP1S-mediated autophagy to remove the accumulated products. These products enhance oxidative stress, triggering DNA double-strand breaks and weakening mitotic checkpoint so that genome instability results after continuous karyotype evolution. Genome instability enhances tumor initiation and development.

Levels of p62 serve as accurate monitors of the intensity of cellular oxidative stress that causes DNA DSB. A defect in autophagy enhances oxidative stress usually resulting in cell death, but instead leads to genome instability when oxidative stress simultaneously subverts mitotic checkpoints and leads to mitotic slippage. Indeed, accumulation of p62 in the MAP1S-deficient mice leads to accumulation of DNA DSB as represented by the levels of phosphorylated H2AX. Chromosomal fragments with breaks tend to fuse with each other and potentially lead to the formation of a new chromosome with two centromeres that will form chromosomal bridges during metaphase and generate new broken ends after telophase. If cells can survive, the resulting aneuploid daughter cells potentially initiate cascade of autocatalytic karyotypic evolution through continuous cycles of chromosomal breakage-fusion-bridge that eventually destabilizes the genomes. A destabilized genome provides opportunities for phenotypic selection which results in continuous evolution and diversity of cancer phenotypes during tumor progression (**Fig. 1B**). Therefore, the MAP1S-deficient mice carrying higher intensities of DNA DSB and genome instability have a higher chance to develop tumor foci.

Hepatocytes carrying DNA DSB get chances to divide several cycles before the liver becomes fully matured. Those progenitor cells remain invisible in liver tissues for several months before developing into tumor foci under a suitable microenvironment. We perform partial hepatectomy to induce liver regeneration in 2-mo-old mice to increase the number of mitotic hepatocytes for study. The MAP1S-deficient mice exhibit higher levels of DNA DSB than the wild types. We further examined the livers of four-month-old DEN-treated mice and found no tumor foci in either wild-type or MAP1S-depleted mice. The MAP1S-depleted mice contained slightly,

but significantly, higher levels of DNA DSB. Examination of DNA contents by flow cytometry revealed that hepatocytes from both wild type and the MAP1S-depleted mice contain amounts of genomic DNA that are deviated from their normal 2N DNA content. The depletion of the *MAP1S* gene causes a wider range of variation of 2N DNA contents and a higher percentage of polyploid hepatocytes. Thus, the knockout mice have higher levels of tumorigenesis-driving genome instability.

To understand why some tumor foci are benign but some others are malignant, we measured the MAP1S levels in adenomas of similar sizes in DEN-treated 10-mo-old mouse livers. The levels of MAP1S were undetectable in nontumor adjacent liver tissues from wild-type mice, but were dramatically elevated in the majority of tumor foci in response to metabolic stresses resulting from genome instability. The MAP1S-deficient mice accumulated higher levels of p62, DNA DSB and genome instability in their tumor foci because of autophagy defects. A higher degree of genomic instability in the tumor foci leads to the development of more malignant HCC.

In summary, MAP1S positively regulates autophagic activity, and its increase upon DEN exposure indicates an autophagic activation. Activated autophagy leads to removal of p62-associated aggresomes and suppression of genome instability immediately upon DEN exposure. The MAP1S-depleted mice accumulate higher levels of genomic instability and have a higher probability to generate tumor progenitor cells that will be developed into visible tumor foci several months later. Within tumor foci, a destabilized genome will reactivate MAP1S-regulated autophagic machinery and in turn reduce the levels of p62 and oxidative stress. MAP1S-depleted mice with higher genomic instability in tumor foci will develop highly malignant hepatocarcinomas. The high incidence of tumor foci and hepatocarcinomas in the MAP1S-depleted mice clearly attest that MAP1S suppress hepatocarcinogenesis through autophagy regulation.

Acknowledgments

This work was supported by DOD New Investigator Award W81XWH (L.L.), NCI 1R01CA142862 (L.L.), NCI P50 CA140388 (W.L.M.), the Susan Komen Foundation (W.L.M.), and aid from the John S. Dunn Research Foundation (W.L.M.).