Autophagy Enhanced by Microtubule- and Mitochondrion-Associated MAP1S Suppresses Genome Instability and Hepatocarcinogenesis

Rui Xie, Fen Wang, Wallace L. McKeehan, and Leyuan Liu

Abstract

Dysfunctional autophagy is associated with tumorigenesis; however, the relationship between the two processes remains unclear. In the present study, we showed that MAP1S levels immediately become elevated in response to diethylnitrosamine-induced or genome instability-driven metabolic stress in a murine model of hepatocarcinoma. Upregulation of MAP1S enhanced autophagy to remove aggresomes and dysfunctional organelles that trigger DNA double-strand breaks and genome instability. The early accumulation of an unstable genome before signs of tumorigenesis indicated that genome instability caused tumorigenesis. After tumorigenesis, tumor development triggered the activation of autophagy to reduce genome instability in tumor foci. We, therefore, conclude that an increase in MAP1S levels triggers autophagy to suppress genome instability such that both the incidence of diethylnitrosamine-induced hepatocarcinogenesis and malignant progression are suppressed. Taken together, the data establish a link between MAP1S-enhanced autophagy and suppression of genomic instability and tumorigenesis.

Introduction

Autophagy, or self-digestion, is a process that begins with the formation of isolation membranes that engulf substrates such as aggregated proteins or damaged organelles to form autophagosomes. During different stages of cell cycle, cells normally retain robust autophagic activity to remove aggregated proteins or damaged organelles such as mitochondria to maintain cellular homeostasis (1). Under nutritive stresses, autophagy is activated to digest cellular organelles or protein aggregates and recycle the basic components for survival (2). An overactivated autophagy causes organelle depletion and type II programmed cell death (3). If the autophagic process is blocked before the stage of autophagosomal formation, the fragmented mitochondria will release cytochrome c and other small molecules to induce conventional apoptosis (4). If autophagosomes are not degraded efficiently, accumulated mitochondria may become damaged by their own production of superoxide and start to leak electrons and will lose their membrane potentials. Thus, diverse forms of aggregation and perinuclear clustering of mitochondria are formed and further induce robust oxidative stress that might also trigger apoptotic cell death (5). Therefore, autophagy not only promotes survival but also controls cell death. Although a link between autophagic malfunction and cancer was established (6-11), the mechanism by which autophagy suppresses tumorigenesis remains largely unknown (2).

MAP1S was originally described and named the chromosome 19 open reading frame 5 (C19ORF5; refs. 12, 13). It is a widely distributed homologue of neuronal-specific MAP1A and MAP1B (13, 14). Similar to MAP1A/B, full-length MAP1S (MAP1SFL) gives rise to multiple posttranslationally modified isoforms, including heavy chain (HC) and light chain (LC) isoforms. Prolonged mitotic arrest or inhibition of the 26S proteasome causes accumulation of an otherwise highly labile short chain (SC) of MAP1S (15, 16). In collaboration with leucine-rich PPR motif-containing protein (LRPPRC) that associates with mitochondria and interacts with Parkinson disease–related mitophagy initiator Parkin, a tumor suppressor and microtubular stabilizer RASSF1A, and LC3 that associates with isolation membrane (12, 13, 16, 17), MAP1S bridges autophagic components with microtubules and mitochondria to affect autophagosomal biogenesis and degradation (16). Ablation of the Map1s gene in mice causes impairment in both basal autophagy for clearance of abnormal mitochondria and nutritive stress–induced autophagy for nutrient recycling (16). The MAP1SSC associates with mitochondria in addition to microtubules and causes irreversible aggregation of dysfunctional mitochondria resulting in mitotic cell death (15). Therefore, the MAP1S-depleted mice serve as a good model to test the relationship between autophagy and tumorigenesis.

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In this study, we show that in response to the acute increase of oxidative stress imposed by exposure to a chemical carcinogen diethylnitrosamine (DEN), MAP1S levels in mouse livers are dramatically elevated to a peak within 1 day and then decrease to an undetectable level 2 days after exposure. The acute elevation of MAP1S levels in mouse livers on DEN exposure leads to activation of autophagy. Activated autophagy leads to removal of p62-associated aggresomes and dysfunctional mitochondria and reduction of DNA double-strand breaks (DSB) and genome instability. Because of the ineffective autophagy machinery in the absence of MAP1S, the MAP1S-depleted mice accumulate higher levels of the p62 and γ-H2AX–marked genome instability even after the acute effect of DEN exposure diminishes 2 days later. The higher levels of genome instability than in wild-type mice remain in the livers of DEN-treated adult MAP1S-depleted mice. After tumor foci are detectable in wild-type mice, MAP1S levels again shift to high levels in tumor foci but remain undetectable in their adjacent nontumor tissues. Elevated levels of MAP1S in tumor foci lead to reactivation of autophagy, further leading to reduction of genomic instability within the tumor foci. Because of higher levels of genome instability in liver tissues before tumorigenesis and tumor foci after formation, the MAP1S-depleted mice develop more tumor foci and those benign adenomas are faster to become more malignant hepatocarcinomas. This is the first report from in vivo studies to show a relationship between the autophagy-suppressed oxidative stress and genome instability–driven tumorigenesis from the earliest origin to the latest stage of tumorigenesis.

Materials and Methods

Reagents and antibodies
Antibody against MAP1S (4G1) recognizing an epitope between human MAP1S sequence D667 and S767 was obtained from A&G Pharmaceutical, Inc. (14, 16). Primary antibodies against β-actin, phosphorylated-H3, FITC–, and rhodamine-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology, Inc. Antibody against p62 (SQSTM1; rabbit polyclonal, BML-PW9860) was obtained from Enzo Life Sciences International, Inc. Antibody against γ-H2AX (rabbit polyclonal, A300-081A) was from Bethyl Laboratories, Inc. The horseradish peroxidase (HRP)-conjugated secondary antibodies were from Bio-Rad. DEN was purchased from Sigma-Aldrich. The ECL Plus Western Blotting Detection System was procured from GE Healthcare Biosciences.

Analysis of DEN-induced hepatocarcinogenesis in mice
Wild-type and MAP1S-depleted mice were created as described (16) and housed in a pathogen-free animal facility under a standard 12-hour light/12-hour dark cycle with ad libitum water and chow. Animal protocols were approved by the Institutional Animal Care and Use Committee, Institute of Biosciences and Technology, Texas A&M Health Science Center. Hepatocarcinogenesis were induced with the hepatocarcinogen DEN as described previously (18). Briefly, cohorts of male 15-day-old wild-type and MAP1S-depleted littermates were subjected to a single intraperitoneal administration of 10 μg/g body weight DEN dissolved in saline. Mice were sacrificed with euthanasia techniques at 4, 6, 8, 10, and 12 months after birth. Immediately after euthanasia, whole-body weights were recorded and the livers were excised, weighed, and photographed. Grossly visible surface liver tumors were scored from the top and bottom of each liver. Large macroscopic lesions and their adjacent normal tissues were dissected out. Tissues were either fixed or frozen in liquid nitrogen immediately and stored at −80°C following standard protocols.

Immunoblot, immunostaining, and fluorescent confocal microscopy
Lysates from normal liver tissues or tumor samples were prepared and immunoblotting was carried out as described (16). Immunostaining was carried out on 5-μm-thick sections mounted on Superfrost Plus slides (Fisher Scientific). Sections were then deparaffinized in xylene and rehydrated through a graded series of ethanol/water solutions. The antigens were retrieved by boiling in citrate buffer (10 mmol/L sodium citrate sodium buffer, pH 6.0) for 20 minutes at 100°C or according to instructions by manufacturers of the antibodies. The specifically bound antibodies were detected with fluorescent- or HRP-conjugated secondary antibody and visualized using the TSA Plus Fluorescence System. The sections were counterstained with TO-PRO-3 iodide to label nuclei and observed under a Zeiss LSM 510 confocal microscope.

To observe autophagosomes in vivo, mouse liver tissues were harvested, frozen, and cryosectioned. As previously reported (16), the GFP-LC3 distribution in frozen sections was directly detected with fluorescent confocal microscopy. The acquired images were converted to 8-bit binary files, and the number, size, and total occupied area of GFP-LC3 punctate foci greater than 4 pixels in diameter on each image were calculated by ImageJ software.

Partial hepatectomy and liver regeneration
Liver regeneration induced by partial hepatectomy and subsequent analysis were done as described previously (19). Briefly, two thirds of the liver was surgically removed from 1.5-month-old male mice (20). Mice were sacrificed 48 hours later. Samples were collected from the regenerated liver tissues and processed for analyses.

Isolation of liver nuclei and flow cytometric analysis
Male mice of indicated age were sacrificed by cervical dislocation. Their livers were rapidly removed and frozen in the liquid nitrogen. The frozen livers were thawed and used to isolate nuclei as described previously (21). For flow cytometric analysis, the nuclei pellet was resuspended in propidium iodide (PI) buffer (50 μg/mL PI and 10 μg/mL RNase in PBS). The resuspended nuclei were set for 30 minutes at 4°C and analyzed by flow cytometry. The individual profiles of each type of mice were superimposed on each other and DNA contents at 2N peaks were measured. The number of nuclei under each peak of 2N to 8N was counted.
Results

Depletion of MAP1S gene accelerates DEN-induced hepatocarcinogenesis in mice

To investigate the tumor suppression function of the MAP1S gene, we set up pairs of wild-type and MAP1S-depleted mice under identical condition without carcinogen and observed for tumorigenesis in all of organs. No abnormality associated with the onset of neoplasia was detected up to the age of 12 months. For example, liver tissues of MAP1S-depleted mice were as healthy as those of wild-type mice (Fig. 1A and B). We reasoned that although depletion of MAP1S may not be sufficient to initiate tumorigenesis, the presence of MAP1S may suppress tumorigenesis induced under certain types of stresses.

We started to induce hepatocarcinogenesis with DEN, a specific initiator of hepatocyte carcinogenesis (18). Littermates of wild-type (+/+ ) and MAP1S-depleted mice (-/-) were

![Figure 1. Depletion of MAP1S promotes DEN-induced hepatocarcinogenesis in mice. A, a summary of tumorigenesis in the liver tissues of wild-type (WT) and MAP1S-depleted mice at different times after DEN treatment. Data are the mean ± SD of each group, and difference was statistically determined by the Student t test; *, P < 0.05. B, the morphology of livers of DEN-treated WT and MAP1S-depleted mice at different ages. (12), 12-month-old mice without DEN treatment. Bar, 10 mm. C, a comparative hematoxylin and eosin (H&E) staining of livers of DEN-treated 6-month-old WT and MAP1S-depleted mice. A normal liver of the WT mouse is compared with a liver with adenomas of MAP1S-depleted mouse. Bar, 100 μm. D and E, a plot of tumor number (D) or ratio of liver weight to body weight (E) to ages of DEN-treated WT and MAP1S-depleted mice as listed in A. F, a comparative H&E staining of livers of DEN-treated 10-month-old WT and MAP1S-depleted mice. Bar, 100 μm. G, a plot of hepatocarcinoma (HCC) incidence to ages of WT and MAP1S-depleted mice after DEN treatments as listed in A. *, P < 0.05.](https://www.aacrjournals.org/cancerres/article-pdf/71/24/6624/26611409/cancerres-A1112170.pdf)
subjected to a single dose of DEN injection at 15 days postnatally when their livers underwent active proliferation. Their livers reached the full sizes of mature livers approximately 2 weeks later. We checked for hepatocarcinogenesis and found tumor foci in MAP1S-depleted liver tissues at 6 months and in wild-type liver tissues at 8 months after birth (Fig. 1A and B). Histologically, the tumor foci were primarily hepatocellular adenomas that caused compression but no disruption of the surrounding normal tissue (Fig. 1C). When mice became older, their body weights were not significantly changed, but tumor incidence, average number of surface tumors per mouse, and ratio of liver weight to body weight (LW/BW) were gradually increased. 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 wild-type mice at the same ages (Fig. 1A, B, D, and E). Therefore, MAP1S is a tumor suppression gene.

Further detailed examination revealed that hepatocellular carcinoma (HCC) was detected 10 months after birth (Fig. 1A and B). The typical trabecular hepatocarcinomas coexisted with the lipid droplet-containing adenomas in aged mice (Fig. 1F), but the MAP1S-depleted mice displayed higher incidence of HCC than the wild-type (Fig. 1A, B, and G). Depletion of MAP1S gene not only promoted the initiation of tumor foci but also accelerated the conversion of hepatocellular adenomas to malignant hepatocarcinomas.

Acute elevation of MAP1S levels immediately upon exposure to DEN leads to activation of autophagy and reduction of DNA DSB

Although the mechanism is not clearly deciphered, autophagy has been hypothesized to reduce chromosomal instability and suppress tumorigenesis through elimination of p62-associated aggregated proteins (7, 8). We attempted to deduce a role of MAP1S-regulated autophagy in tumor suppression. The expression levels of MAP1S in normal hepatocytes were too low to be detected by immunoblotting, but were dramatically elevated immediately upon exposure to DEN, and then reduced to an undetectable level 2 days after exposure to DEN (Fig. 2A). Our previous results have suggested that MAP1S levels increase on autophagic activation (16). The wild-type accumulated fewer autophagosomes labeled with GFP-LC3 punctate foci in hepatocytes than MAP1S-depleted mice (Fig. 2B and C), indicating that the acute elevation of MAP1S caused activation of both autophagy initiation and autophagosomal degradation. Examination of LC3-II levels representing a balance of autophagosomal generation and degradation at each sampling point in detail revealed that acute elevation of MAP1S on DEN exposure enhanced both events but still did not efficiently process all autophagosomes such that a certain amount of autophagosomes accumulated and reached a peak at 12 hours. The MAP1S-deficient mice may generate autophagosomes slowly such that LC3-II levels reached a peak at least 12 hours later than the wild-type mice; in addition, they less efficiently remove autophagosomes through lysosomes such that LC3-II levels dropped to base levels 5 days later than in the wild-type mice (Fig. 2A and D).

MAP1S and GFP-LC3 signals distributed in cells in a mutually exclusively manner, indicating that autophagosomes had been degraded efficiently in cells with elevated levels of MAP1S. Approximately 40% of cells had enhanced levels of MAP1S and activated autophagy to degrade autophagosomes whereas the other 60% cells did not have increased levels of MAP1S to degrade autophagosomes. More than 80% of hepatocytes accumulated autophagosomes in the MAP1S-depleted mice (Fig. 2E and F), further confirming the role of MAP1S in autophagy. Because of active autophagy, the wild-type hepatocytes were able to efficiently eliminate the p62-associated aggresomes and dysfunctional organelles. Two weeks after exposure, levels of p62 reduced to levels close to but still higher than those before exposure. In contrast, the MAP1S-deficient hepatocytes maintained higher levels of p62 than the wild-type mice (Fig. 2A and D). Therefore, elevated levels of MAP1S lead to activation of autophagy to reduce the DEN-induced aggresomes and dysfunctional organelles.

Protein aggresomes and dysfunctional organelles such as mitochondria and endoplasm reticulum induce oxidative stress that causes DNA DSB and genome instability (7, 8, 22). Intensity of DNA DSB is represented by the levels of phosphorylated H2AX (γ-H2AX; ref. 23). No DNA DSB labeled with γ-H2AX was found in untreated hepatocytes of both wild-type and MAP1S-depleted mice (Fig. 2G). Together with the acute elevation of MAP1S levels, DNA DSB was also dramatically intensified immediately on exposure to DEN (Fig. 2A, D, and H). Similar to the mutually exclusive distribution of GFP-LC3 punctate foci and MAP1S signals, MAP1S levels were lower in the cells with DNA DSB (Fig. 2G). The MAP1S-depleted mice exhibited higher levels of DNA DSB in hepatocytes immediately on DEN treatment and maintained higher levels than the wild-type mice until livers became fully mature 2 weeks after DEN treatment (Fig. 2A, D, and H). Therefore, depletion of the MAP1S gene impaired normal autophagy function, leading to accumulation of higher levels of genome instability that increase the chance to form tumor foci in the future.

The DEN-induced DNA DSB and genome instability that persist in the adult liver tissues are suppressed by MAP1S

It is well known that chromosomal fragments with DSB tend to randomly fuse either correctly with the original broken chromosomal fragment or erroneously with a fragment broken from another chromosome. The erroneously ligated chromosome undergoes breakage–fusion–bridge cycles in successive mitoses that eventually lead to chromosome instability (24). Majority of hepatocytes with DNA DSB on DEN treatment had their broken ends fused such that no γ-H2AX signal could readily be detected 1 month after exposure to DEN. We hypothesized that those hepatocytes could still generate daughter cells with DNA DSB because of the breakage–fusion–bridge mechanism. However, nearly no mitotic hepatocyte could be identified in the adult livers (Fig. 3A); therefore, that it was technically difficult to test the hypothesis. To increase the mitotic index, we conducted partial hepatectomy to induce liver regeneration in 2-month-old mice, which led to a dramatic increase in the number of mitotic hepatocytes (Fig. 3A).
Figure 2. Acute elevation of MAP1S levels immediately on DEN exposure leads to activation of autophagy and reduction of DNA DSB. A, an immunoblot analysis of liver lysates from WT (+/+) and MAP1S-depleted mice (−/−) injected with DEN (25 μg/g body weight). Liver tissues were collected immediately or on different days after DEN injection. FL, full-length MAP1S; HC, heavy chain of MAP1S. B, representative images showing GFP-LC3 punctate foci that appeared in liver tissues at the indicated times after DEN treatment. The GFP-LC3+/− MAP1S+/− mice (−/−) and their GFP-LC3+/− MAP1S−/− controls (+/+) were similarly treated with DEN as in A. Bar, 100 μm. C, quantitation of the total area of GFP-LC3 punctate foci with size larger than 100 pixel². Data are the average and SD of 10 randomly selected images in a field of 512 × 512 pixel². The significance of differences was determined by the Student t test; *, P < 0.05. D, a plot of relative intensities of p62 and γ-H2AX bands shown in A with the value in WT mice at day 0 as the standard 1. Data are a representative set of 3 repeats, all of which exhibit the same large differences between the WT and knockout (KO) but varied relative intensities due to the varied intensity of the standards. E, relative distribution of GFP-LC3 and MAP1S in liver tissues 12 hours after DEN treatment. Tissue sections prepared as in B were fixed and subjected to immunostaining. Cell boundaries were outlined with white dot lines. Bar, 20 μm. F, percentage of cells with MAP1S-positive signals or GFP-LC3 punctate foci 12 hours after DEN treatment. Samples were prepared and observed as described in E. Data are the mean of 10 randomly selected images containing approximately 2,000 cells in total. G, relative distribution of MAP1S and γ-H2AX in liver tissues 12 hours after DEN treatment. Pairs of MAP1S+/− (+/+) and MAP1S−/− mice (−/−) were similarly treated with DEN as in A, and liver tissues were fixed and subjected to immunostaining. Bar, 10 μm. H, representative images showing γ-H2AX signals in liver tissues of MAP1S+/− (+/+) and MAP1S−/− mice (−/−) at the indicated times after DEN treatment. Nuclei were counterstained. Bar, 100 μm.
Both normal and DEN-treated mitotic hepatocytes were induced into mitosis during liver regeneration. The untreated mitotic hepatocytes exhibited no DNA DSB, whereas mitotic hepatocytes that had been exposed to DEN 1.5 months previously contained a large number of DNA DSB (Fig. 3B). The DNA DSB signals were seen to clearly overlap with the condensed chromosomes in the mitotic hepatocytes (Fig. 3C).

Relatively, fewer mitotic hepatocytes carried DNA DSB in the wild-type than in the MAP1S-depleted mice (Fig. 3B and D). Although partial hepatectomy created a highly artificial and unexpected biologic condition, the results indicated that hepatocytes in DEN-treated MAP1S-depleted mice had a higher chance than the wild-type to generate daughter hepatocytes with genome instability if they had a chance to re-enter mitosis.

We further examined the livers of 4-month-old DEN-treated mice and found no tumor foci in either wild-type or MAP1S-depleted mice (Fig. 1A). Constant evolution of the destabilized genomes during the 3.5 months after exposure to DEN might lead to preservation of DNA DSB although no tumor focus was detectable. The MAP1S-depleted mice contained slightly but significantly higher levels of DNA DSB in livers that did not develop into any detectable tumor focus yet (Fig. 4A and B). Examination of DNA contents by flow cytometry revealed that hepatocytes from both wild-type and the MAP1S-depleted mice contained amounts of genomic DNA that had deviated from their normal 2N DNA content (Fig. 4C). The depletion of MAP1S gene caused a wider range of variation of 2N DNA contents (Fig. 4C and D) and had a higher percentage of polyploidy hepatocytes (Fig. 4C and E). Polyploid cells often lead to generation of aneuploidy and genome instability during proliferation because of weakened mitotic checkpoint (25). Thus, the knockout mice had higher levels of tumorigenesis-driving genome instability.

**Elevation of MAP1S levels within tumor foci of DEN-treated mice leads to activation of autophagy influx and reduction of DNA DSB**

The persistence of higher genomic instability in the MAP1S-depleted hepatocytes represented a higher chance of tumorigenesis, which explains why the MAP1S-depleted mice had higher tumor incidence and more surface tumors than the wild-type mice. However, the reason for the MAP1S-depleted mice developing more malignant HCC was not explained. We measured the MAP1S levels adenomas of similar sizes in DEN-treated 10-month-old mouse livers. The levels of MAP1S were undetectable in non–tumor-adjacent liver tissues from wild-type mice, and were similar to the levels in liver tissues of untreated mice (Fig. 2A), but dramatically elevated in the
majority of tumor foci (Fig. 5A–C). It was likely that such increase was caused by metabolic stresses that resulted from genome instability.

Cells in the aged livers contained different genomes among different individuals (Fig. 4C and D). Each diversified genome evolves and adopts a different set of autophagy machinery. Under such conditions, the LC3-II levels may represent balances of different generation and degradation rates. As expected, the LC3-II levels were not significantly different between tumor foci and neighboring tissues, and between wild-type and MAP1S-depleted mice (Fig. 5C and D). However, high levels of MAP1S indicate activation of autophagy in flux leading to reduction of autophagy substrates (16). More p62-associated protein aggregates and dysfunctional organelles accumulated in MAP1S-deficient tumor foci (Fig. 5C, E, and F), indicating dramatic reduction of autophagy influx. The increase of MAP1S levels leads to increase of autophagy influx and reduction of p62 levels in the DEN-induced tumor foci of wild-type livers. The deficiency in autophagic response in the tumor foci of the MAP1S-depleted livers reflected by such accumulation correspondingly led to increase of DNA DSB (Fig. 5C, G, and H). Higher degree of genomic instability in the tumor foci led to development of more malignant HCC. Thus, an active autophagy suppressed genome instability in tumor foci such that a less malignant tumor was developed.

Discussion

Chromosomal instability, as the most common type of genome instability, mainly results from either whole-chromosome aneuploidy due to mitotic errors or segmental...
aneuploidy due to chromosomal rearrangements including deletions, amplifications, or translocation (26). Although aneuploidy is not synonymous with chromosomal instability (26), aneuploidy was hypothesized to be the origin of tumorigenesis (27). Since then, it has been strongly debated whether point mutation or genomic instability is the cause or a consequence of tumorigenesis (28). Our results indicate that, early before tumorigenesis, significant levels of genomic instability exist in the liver tissues exposed to the chemical carcinogen DEN. This indicates that genomic instability may be the cause of hepatocarcinogenesis. The positive correlation between genomic instability and malignancy of tumor foci as revealed in this study further indicates that genomic instability is also a driving force in cancer development. Thus, high levels of genomic instability provide high chances of tumor initiation and wide genetic variation to evolve into highly malignant tumor foci.

An inverse relationship between autophagic activity and malignant transformation has been established since 1999 (26). Although aneuploidy is not synonymous with chromosomal instability (26), aneuploidy was hypothesized to be the origin of tumorigenesis (27). Since then, it has been strongly debated whether point mutation or genomic instability is the cause or a consequence of tumorigenesis (28). Our results indicate that, early before tumorigenesis, significant levels of genomic instability exist in the liver tissues exposed to the chemical carcinogen DEN. This indicates that genomic instability may be the cause of hepatocarcinogenesis. The positive correlation between genomic instability and malignancy of tumor foci as revealed in this study further indicates that genomic instability is also a driving force in cancer development. Thus, high levels of genomic instability provide high chances of tumor initiation and wide genetic variation to evolve into highly malignant tumor foci.

Figure 5. Elevation of MAP1S levels leads to activation of autophagy influx and reduction of DNA DSB within tumor foci in livers of DEN-treated 10-month-old mice. A, representative images showing the immunostaining patterns of MAP1S in adjacent tumor tissues (T) and normal tissues (NT) from livers of DEN-treated 10-month-old WT (+/+) and MAP1S-depleted mice (−/−). All bars in this figure are equal to 100 μm. B, a summary of percentage of MAP1S-positive tumor foci in livers of WT (+/+) and MAP1S KO mice (−/−). Approximately 10 hepatoadenomas were examined for each group. C, immunoblot of lysates from adjacent tumor tissues (T) and normal tissues (NT) isolated from livers of DEN-treated 10-month-old WT (+/+) and MAP1S-depleted mice (−/−). The same amount of total proteins was loaded for each lane. D, a plot of relative intensities of LC3-II bands shown in C with the value in the first WT tumor sample as the standard. Data are the mean and SD of 3 repeats. E, representative images showing immunostaining profiles of liver tissues carrying hepatoadenoma with antibody against p62. F, a plot of relative intensities of p62 bands shown in C with the value in the first WT tumor sample as the standard. Data are the mean and SD of 3 repeats. The significances of differences between tumor and adjacent normal tissues and between WT and KO mice were determined by the Student t test; *, P < 0.05. G, representative images showing immunostaining profiles of liver tissues carrying hepatoadenoma with antibody against γ-H2AX. H, a plot of relative intensities of γ-H2AX bands shown in C. Data were collected and analyzed similarly as in F.
intensity of cellular oxidative stress. A defect in autophagy enhances oxidative stress resulting in cell death (7). However, oxidative stress leads to DNA DSB (22, 33, 34) because it can simultaneously subvert mitotic checkpoints (35, 36).

From a cytogenetic perspective, a chromosomal fragment with a break tends to randomly fuse with another broken chromosomal fragment, which potentially leads to the formation of a new chromosome with 2 centromeres if ligation occurs between 2 fragments with centromeres. The cell containing the chromosome with 2 centromeres forms a chromosomal bridge during mitotic metaphase and the resultant chromosomal bridge will break and generate new broken ends after telophase (24, 37). Modern molecular biology indicates that DNA DSB will be repaired through either homologous recombination (HR) or nonhomologous end-joining (NHEJ), whereas NHEJ is believed to be the predominant mechanism of DSB repair in higher eukaryotes (38). Cells with DSBs will experience a mitotic defect that will activate mitotic checkpoint and temporally result in mitotic arrest and mitotic cell death (15). If cells can escape the arresting through the so-called mitotic slippage, the resultant aneuploid daughter cells are generally lethal. Therefore, genome instability is intrinsically tumor suppressive (39). However, if an aneuploid cell can escape the mitotic checkpoint and survive, it potentially initiates a cascade of autocalytic karyotypic evolution through continuous cycles of chromosomal breakage–fusion–bridge and eventually destabilizes the genome. A destabilized genome results in either inactivation of tumor suppressor genes or activation of oncogenes through point mutation, rearrangement of wild-type genes in new locations on genome to create malignancies, or creation of fusion gene between different wild-type genes to fulfill a new function. Such genetic variation generates the opportunity for phenotypic selection, which results in continuous evolution and diversity of cancer phenotypes during the progression (24, 28, 40). Thus, autophagic defect-resultant oxidative stress could either suppress tumorigenesis if it can trigger cell death or promote tumorigenesis if the cells can survive the insult of oxidative stress.

It has been known that animals exposed to DEN exhibit increased levels of oxidative stress (41). We believe that such increase in oxidative stress may be caused by accumulation of p62-associated protein aggresomes and dysfunctional organelles. We knew that MAP1S positively regulates autophagic activity and its increase on DEN exposure indicates an autophagic activation. Activated autophagy leads to removal of p62 and reduction of oxidative stress while animals with defective autophagy accumulate high levels of p62 and oxidative stress. MAP1S-activated autophagy causes reduction of both oxidative stress and genomic instability immediately on DEN exposure. The MAP1S-depleted mice accumulate higher levels of genomic instability and have higher probability to generate tumor progenitor cells that will be developed into visible tumor foci several months later. For the tumor progenitor cells to evolve into large tumor foci, cells continue multiple rounds of mitoses that will further destabilize the genome and results in accumulation of oxidative stress (42). Such stress will reactivate MAP1S-regulated autophagic machinery and, in turn, reduce the levels of p62 and oxidative stress. The inactive autophagy in MAP1S-depleted mice causes accumulation of p62, oxidative stress, and genomic instability in tumor foci. Mice with a destabilized genome will develop highly malignant hepatocarcinomas. The high incidence of tumor foci and hepatocarcinomas in the MAP1S-depleted mice clearly attest that MAP1S suppresses hepatocarcinogenesis through regulation of autophagy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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