N-Methyl-N-nitrosourea, an N-nitroso compound converted from dietary nitrate by *Helicobacter pylori*, causes somatic mutations in epithelial cells and induces gastric premalignancy. Here, we describe a detailed protocol for induction of gastric tumor and analysis of tumor phenotypes in mice. This model can be widely used for studying the initiation and growth of gastric cancer.
Protocol for chemically induced murine gastric tumor model

Ke Li,1,2,* Ao Wang,1 Huijuan Liu,1 and Baojie Li1,3,*

1Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University, Shanghai 200240, China
2Technical contact
3Lead contact
*Correspondence: libj@sjtu.edu.cn (B.L.), zlkeli@sjtu.edu.cn (K.L.)
https://doi.org/10.1016/j.xpro.2021.100814

SUMMARY
N-Methyl-N-nitrosourea, an N-nitroso compound converted from dietary nitrite by Helicobacter pylori, causes somatic mutations in epithelial cells and induces gastric premalignancy. Here, we describe a detailed protocol for induction of gastric tumor and analysis of tumor phenotypes in mice. This model can be widely used for studying the initiation and growth of gastric cancer.
For complete details on the use and execution of this protocol, please refer to Li et al. (2021).

BEFORE YOU BEGIN
The protocol below describes the specific steps for wild-type and Tsc1-deficient C57BL/6 mice. However, we have also used the protocol for Mek1-deficient and Bmpr1a-deficient mouse lines. The animal experiments in this protocol have followed the recommendations of the National Research Council Guide for Care and Use of Laboratory Animals. All protocols have been approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University, China.

Note: Gastric tumor models have been generated in other mouse strains including FVB, BALB/c, C3H/He and MSM (Table 1).

Prepare Lgr5-GFP-CreERT; Tsc1ff mice

© Timing: more than 6 months

1. Purchase the Lgr5-GFP-CreERT mice and Tsc1ff mice from the Jackson Laboratories and breed them to the fertile age.
2. Cross Lgr5-GFP-CreERT mice with Tsc1ff mice.
3. Genotype the offspring. Mouse tails are lysed in a lysis buffer containing Proteinase K (2%) and genomic DNA is extracted with isopropanol precipitation. We wash the DNA pellets with 70% ethanol and dry the pellet. The DNA is used for genotyping with PCR.

Note: The first filial generation is Lgr5-GFP-CreERT; Tsc1ff and we need cross these mice to generate Lgr5-GFP-CreERT; Tsc1ff mice.
Table 1. Tumor formation induced by MNU in various organs of different mouse strains

| Tumors of organ | Treatment | Strains of mouse | Concentration and administration* | Time of MNU treatment | Duration after MNU treatment | Incidence | Reference |
|-----------------|-----------|------------------|------------------------------------|-----------------------|-----------------------------|-----------|-----------|
| Stomach         | MNU       | C57BL/6          | 240 ppm, d.w                       | 5 weeks               | 22 weeks                    | 60%       | Li et al. (2021) |
|                 | MNU       | C56BL/6, FVB     | 240 ppm, d.w                       | 5 weeks               | 26 weeks                    | 90%       | Tomita et al. (2011) |
|                 | MNU       | C57BL/6          | 120 ppm, d.w                       | 5 weeks               | 40 weeks                    | 80%       | Yamamoto et al. (2000) |
|                 | MNU       | C57BLKS          | 60 ppm, d.w                        | 30 weeks              | 0 weeks                     | 57%       | Yoshizawa et al. (2009) |
|                 | H. pylori | C57BL/6, FVB     | 240 ppm, d.w                       | 5 weeks               | 26 weeks                    | 100%      | Tomita et al. (2011) |
| Kidney          | MNU       | BALB/c           | 120 ppm, d.w                       | 10 weeks              | 30 weeks                    | 54%       | Yamachika et al. (1998) |
|                 | MNU       | BALB/c           | 60 ppm, d.w                        | 10 weeks              | 30 weeks                    | 58%       | Yamachika et al. (1998) |
|                 | MNU       | C3H/He           | 120 ppm, d.w                       | 20 weeks              | 30 weeks                    | 58%       | Yamachika et al. (1998) |
|                 | MNU       | C3H/He           | 60 ppm, d.w                        | 20 weeks              | 30 weeks                    | 60.7%     | Tatematsu et al. (1993) |
|                 | MNU       | C3H/He           | 30 ppm, d.w                        | 20 weeks              | 30 weeks                    | 63%       | Tatematsu et al. (1993) |
|                 | MNU       | MSM              | 0.03 mg/g, i.g                     | 10 weeks              | 36 weeks                    | 6.3%      | Masui et al. (1997) |
| Intestine       | MNU       | C57BL/6          | 240 ppm, d.w                       | 5 weeks               | 20 weeks                    | 0%–6.3%   | Ogawa et al. (2013) |
| (duodenum)      | MNU       | C57BL/6          | 50 mg/kg, i.p                      | 1 time                | 20–30 weeks                 | 14%       | Qin et al. (2000) |
|                 | MNU       | C57BL/6          | 50 mg/kg, i.p                      | 1 time                | 30–40 weeks                 | 56%       | Qin et al. (2000) |
| Colon           | H. pylori | C57BL/6J         | 240 ppm, d.w                       | 5 weeks               | 70 weeks                    | 85%       | Ogawa et al. (2013) |
| Lung            | MNU       | C57BL/6          | 240 ppm, d.w                       | 5 weeks               | 20 weeks                    | 11%–25%   | Ogawa et al. (2013) |
|                 | MNU       | BALB/c           | 60 ppm, d.w                        | 6 weeks               | 20 weeks                    | 50 weeks  | Faustino-Rocha et al. (2015) |
|                 | MNU       | A/J              | 50 mg/kg, i.p                      | 4 weeks               | 32 weeks                    | N/A       | Westcott et al. (2015) |
| Bladder         | MNU       | C3H/He           | 7.5 mg/mL, i.ves                   | 1 time                | 4 weeks                     | 28%       | Soloway et al. (1983) |
| Mammary gland   | MNU       | BALB/c           | 5 mg/100g, i.p                     | 3 doses 1 month       | 26 weeks                    | 15%       | Pazos et al. (1992) |
| Kidney          | MNU       | C57BL/6          | 240 ppm, d.w                       | 5 weeks               | 20 weeks                    | 5%–12%    | Ogawa et al. (2013) |
| Liver           | MNU       | C57BL/6          | 240 ppm, d.w                       | 5 weeks               | 20 weeks                    | 18%–22%   | Ogawa et al. (2013) |

* d.w represents drinking water; i.g represents intra gastric intubation; i.p represents intraperitoneal injection; i.ves represents intravesical injection.

---

**Primers for genotyping**

| Primer | Sequence |
|--------|----------|
| Primer 8060 | 5'-CTGCCTCTGCTGCCAGTCCT-3' |
| Primer 8061 | 5'-ATACCCCATCCCCCTTGGAGC-3' |
| Primer 9402 | 5'-CACCCTGGTAAGCAAGCTC-3' |
| Primer 4008 | 5'-GTCAAGACCCTAGGAGAACG-3' |
| Primer 4009 | 5'-GAATCAACCCACAGAGCAT-3' |

**PCR reaction system for Lgr5 knock-in allele**

| Reagent | Amount |
|---------|--------|
| Primer 8060 | 0.6 µL |
| Primer 8061 | 0.8 µL |
| Primer 9402 | 0.4 µL |
| ddH2O | 3.7 µL |
| 2x Taq Mix Buffer | 6.5 µL |
| Sample DNA | 1 µL |
| Total | 13 µL |

**PCR reaction system for Tsc1 floxed allele**

| Reagent | Amount |
|---------|--------|
| Primer 4008 | 0.5 µL |
| Primer 4009 | 0.5 µL |
| ddH2O | 9.5 µL |

(Continued on next page)
Cre/Lox P system working

**Timing:** 3 days

4. Each male mouse received intraperitoneal injection of tamoxifen (TAM) (2 mg/20 g body weight) for 3 consecutive days at 2 months of age.

**Note:** TAM can be dissolved in corn oil and the concentration should be low, due to possible damage to the stomach at high concentrations.

**Note:** TAM should be injected two weeks before starting tumor induction.

**Optional:** for H. pylori preparation, it can be grown on trypticase broth agar medium containing 5% defibrinated sheep blood with microaerobic atmosphere (3–5% O₂ and 10% CO₂) at 37°C. The bacteria are harvested after 48 hrs of growth and re-suspended in trypticase broth for subsequent use.

**KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Chemicals, peptides, and recombinant proteins | | |
| Proteinase K | Millipore | Cat #539480 |
| Tamoxifen | Sigma | Cat #T5648 |
| Corn oil | Aladdin | Cat #C116023 |
| N-Methyl-N-nitrosourea (MNU) | Macklin | Cat #684-93-S |
| 2X Taq Mix buffer | Abmgood | Cat #G013 |

(Continued on next page)
### MATERIALS AND EQUIPMENT

| REAGENT OR RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Ketamine            | Sigma  | Cat #K-002 |
| Xylazine             | Sigma  | Cat #X1126 |
| EDTA                 | Sangon Biotech | Cat #60-00-4 |
| NaCl                 | Sangon Biotech | Cat #7647-14-5 |
| Tris                 | Vetec  | Cat #77-86-1 |
| K2HPO4               | BBI    | Cat #7778-77-0 |
| Na2HPO4-12H2O        | Sangon Biotech | Cat #7782-95-6 |
| KCl                  | Sangon Biotech | Cat #7447-40-7 |
| SDS                  | BBI    | Cat #151-21-3 |
| Ethanol              | Sinopharm | Cat #10009218 |
| Isopropanol          | Sinopharm | Cat #80109218 |
| Dimethyl benzene     | Lingfeng | Cat #1330-20-7 |
| 2,2,2-Tribromoethanol| Sigma  | Cat #T48402 |
| 2-Methyl-2-butanol   | Sigma  | Cat #471712 |
| HCl                  | Lingfeng | Cat #6747-01-0 |
| Parafomaldehyde      | Macklin | Cat #P804537 |
| Paraplast High Melt  | Leica  | Cat #39601095 |
| Tryptone             | Sangon Biotech | Cat #A505250 |
| Soytoye              | Sangon Biotech | Cat #A600214 |
| Trypticase Soy Broth (TSB), Bottled Broth| Sigma | Cat #2699195 |
| U0126                | Selleck | Cat #s1102 |
| LDN-193189           | Selleck | Cat #s2618 |

**Experimental models: organisms/strains**

**Mouse:** Lgr5-GFP-Cre<sup>ERT2</sup> (C57BL/6, male, 2 month-old)  
Dr. Hans Clever, Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW), the Netherlands  
N/A

**Mouse:** Tsc1<sup>loxP/loxP</sup> (C57BL/6, male, 2 month-old)  
The Jackson Laboratories  
JAX: 005680

**Organism:** Helicobacter pylori  
ATCC  
Cat #49179

**Oligonucleotides**

**Primer 8060:** 5’-CTGCTCTCTGCTCCCAGTCT-3’  
This paper  
N/A

**Primer 8061:** 5’-ATACCCCATCCCTTTTGAGC-3’  
This paper  
N/A

**Primer 9402:** 5’-CACCCCGGTGAACAGCTC-3’  
This paper  
N/A

**Primer 4008:** 5’-GTCACGACCGTAGGAGAAGC-3’  
This paper  
N/A

**Primer 4009:** 5’-GAATCAACCACAGAGCAT-3’  
This paper  
N/A

**Other**

| Item                          | Source           | Identifier |
|-------------------------------|------------------|------------|
| Aluminum foil                 | Aka              | Cat #8011-O |
| 8-Strip PCR tube              | LABTIDE          | Cat #P01-0803C |
| 8-Strip flat cap              | LABTIDE          | Cat #P01-0803B |
| Microtubtes (1.5 mL)          | Axygen           | Cat #MCT-150-C |
| General surgical scissor      | RWD Life Science | Cat #514001 |
| General surgical tweezor      | RWD Life Science | Cat #F11020 |
| Disposable syringes (1mL)     | KDL, Shanghai   | Cat #KDL-1mL |
| Sterile gummmed tape          | 3M               | Cat #1322 |
| Tissue Embedding Cassettes    | Xiuwei Commerce  | Cat #BMH-002 |
| Sterile Cotton Swabs          | Medicomp         | Cat #4215352 |
| Water bottles for mouse cages | Baoy, Beijing   | Cat #By100  |

**MATERIALS AND EQUIPMENT**

**Mice tail lysis buffer**

| Reagent          | Final concentration | Amount |
|------------------|---------------------|--------|
| Tris-HCl (pH 8.0)| 1 M                 | 100 mL |
| SDS              | 10% (w/v)           | 40 mL  |

(Continued on next page)
**Note:** Mouse tail lysis buffer may suffer from salting out at 4°C. However, it can be used after brief heating and re-dissolving.

**PBS buffer**

| Reagent          | Final concentration | Amount |
|------------------|---------------------|--------|
| NaCl             | 137 mM              | 8 g    |
| KH$_2$PO$_4$     | 1.47 mM             | 0.24 g |
| Na$_2$HPO$_4$·12H$_2$O | 10 mM             | 3.58 g |
| KCl              | 2.7 mM              | 0.2 g  |
| ddH$_2$O         | N/A                 | 1000 mL|
| Total            | N/A                 | 1000 mL|

Sterilized by autoclaving and store at 24°C–25°C.

**Trypticase broth/Agar**

| Reagent          | Final concentration | Amount |
|------------------|---------------------|--------|
| Tryptone         | N/A                 | 15 g   |
| Soytone          | N/A                 | 5 g    |
| NaCl             | 86 mM               | 5 g    |
| Dextrose         | 14 mM               | 2.5 g  |
| Agar             | N/A                 | 15 g   |
| ddH$_2$O         | N/A                 | 1000 mL|
| Total            | N/A                 | 1000 mL|

Sterilize and store at 4°C. For trypticase broth, omit agar.

**Alternatives:** Commercial trypticase broth medium can be purchased (see key resources table).

**Other materials**

| Name        | Reagents                                                                 |
|-------------|--------------------------------------------------------------------------|
| Tamoxifen   | 10 mg/mL in corn oil. Mix one night and store at 4°C                      |
| Proteinase K| 100 mg dissolved in 5 mL sterilized water                                |
| MNU         | Dissolved to 240 mg/L in sterilized drinking water                       |
| Avertin     | 5 g 2,2,2-Tribromoethanol dissolved in 3.1 mL 2-Methyl-2-butanol for storage at 4°C. Diluted 40 times for administration |
| PFA         | 4 g paraformaldehyde dissolved in 100 mL sterilized water               |

**STEP-BY-STEP METHOD DETAILS**

N-methyl-N-nitrosourea (MNU), a carcinogenic agent, generates somatic mutations in epithelial cells of the stomach and induces tumor formation. In this article, we show that the standard tumorigenesis protocol using MNU (240 ppm) can generate gastric tumors at high incidence in the antrum.

**Note:** Although MNU can be used to induce intestine and colon tumors, the ways of drug administration are quite different. Mice are usually injected with MNU by intraperitoneal
administration for intestine tumor induction and by intrarectal administration for colon tumor induction.

MNU solution preparation

⏱ Timing: 10 min

This step allows you to dissolve MNU in drinking water.

1. Use a light-shielded bottle or cover a transparent one with appropriate size of aluminum foil.
2. Weigh 0.012 g MNU powder on a precise analytical balance under a light-shield condition.
3. Place the MNU into the bottle prepared in step 1.
4. Dissolve MNU in 50 mL sterile drinking water.

⚠️ CRITICAL: Due to instability of MNU, we suggest preparing small amounts of the solution (50 ml) and change it periodically.

**Note:** MNU is a toxic carcinogen and photodecomposition occurs easily. Be very careful when handling it and use appropriated personal protective equipment.

**Note:** The concentration of MNU in drinking water is 240 ppm. It is known that more time is needed for lower concentrations of MNU to induce gastric tumors (Table 1).

MNU treatment

⏱ Timing: 10 weeks

**Optional:** To mimic the microenvironment of human stomach, mice can be infected with *H. pylori* in 0.2 ml trypticase broth by oral gavage 3 times per week, 2 weeks before MNU administration. The total dose of *H. pylori* is around 100 million colony-forming units per mouse (Figure 1A). The dose can be quantified by counting the bacteria on slides under microscope or routine culture plate counting method. *H. pylori* in the gastric gland can be detected by Warthin–Starry staining.

Tumorigenesis in murine stomach relies on oncogenic cues. Here, mice are exposed to MNU, a carcinogen generated by *H. pylori*, in the chemical-induced model (Figures 1A–1D).

**Note:** We recommend using mice at 2–3 months of age. We keep 4 mice in one cage.

5. Place the light-shielded water bottle containing MNU on the cage so the mice have free access.
6. Two days later, replace the MNU solution with newly prepared solution.

**Note:** Repeat the steps 1–4 in the end of step 6. Due to instability of MNU, we suggest the frequency of change is thrice per week or every other day to keep the potency of the agent.
7. After one week of MNU treatment, take out the light-shielded bottle and change it for the normal transparent bottle containing just drinking water.

8. One week later, the transparent bottle should be changed for the light-shielded bottle containing MNU solution.

△ CRITICAL: Steps 5–8 comprise one cycle (2 weeks). Repeat the treatment for 5 cycles in wild-type or TAM-induced genetically modified mice.

9. Repeat step 5 to step 8 for 4 more cycles (Figure 1A).

Free feeding and drinking

△ Timing: approximately 22 weeks

10. Mice are housed in a pathogen-free facility with free access to food and water waiting for the development of gastric tumors, for approximately 22 weeks.

Note: The feeding period after treatment with MNU is flexible and the mice can be sacrificed from weeks 18–26. In addition, the feeding time with low concentration MNU (60 or 120 ppm) should increase to 32–40 weeks.

Optional: The feeding time is an appropriate period for other treatments. Mice can be administered with inhibitors, such as U0126 and LDN-193189, MEK1 and BMPR1A inhibitors respectively, or other drugs via intraperitoneal injection every other day (Figure 1C).

Tumor tissue collection and subsequent process

△ Timing: 3 days

Collect the stomach tissues, take photos of tumors and perform H&E or immuno-staining.

11. Anesthetize the mice using 40 mg/mL Avertin (240 mg/kg body weight) by intraperitoneal injection.

Alternatives: In addition to Avertin, mice can be anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) via intraperitoneal injection.

12. Lay down the animal and fix it on the surgical platform, remove the ribs and carefully expose the heart avoiding the rupture of other tissue, especially the major blood-vessels near the heart.

13. Perfuse sterilized PBS to flush out blood from the heart after removing the auricle (Methods video S1).

△ CRITICAL: During perfusion, inject the PBS slowly at a constant speed. The color of lungs and livers should become light, indicating the loss of blood after a successful perfusion.

14. After perfusion, carefully harvest the stomach and then open the organ along the great curve, wash the tissue with cold PBS three times to remove the food debris (Figure 2A).

15. Unfold the stomach on a white platform or paper to expose the interior of forestomach, corpus, and antrum, and take photos (Figure 2B). Next, quantify the number of tumors in each animal and calculate their volume using a caliper.

Note: The number of tumors is around 2 and the size is from 0.8 to 12 mm³ in wild-type mice. In some genetically modified mice, the size is up to 80 mm³ (Figure 2E).
16. Cut the tumor tissues into several pieces. One part is for histopathological analysis and other parts are frozen for future RNA or protein extraction.

17. Place the tissue into 4% PFA solution to fix it one night and transfer to 70% ethanol for histological analysis.

---

**Pause point:** Tissues can be kept in 70% ethanol at 4°C for a short period of time (1 week maximal) before proceeding to the next step.
18. Perform the routine dehydrating and paraffin embedding procedures for H&E staining.
   a. Transfer tissues from PFA solution to 70% ethanol for 1 h.
   b. Dehydrate the tissues in increasing concentrations of ethanol, 80%, 90%, 95%, and 100% for 1 h each.
   c. Immerse the tissues in dimethyl benzene (100%) for derosination and transparency enhancement for 1 h and then wax them for 3 h. The paraffin should be changed once every hour.
   d. Embed the tissue in paraffin blocks and cut them into 4–5 μm sections for H&E staining or other histological staining.

Optional: Between dehydration and dimethyl benzene treatment, tissues can be transferred to a mix of absolute ethanol and dimethyl benzene for 1 hour, as an intermediate stage.

Note: The histologic classification of tumors can be defined by H&E staining. The sign of hyperplasia is the dysplasia of gastric epithelium with elongation of gland units, and the advanced tumor with glandular and cellular distortion is considered to be adenoma (Figure 2D).

EXPECTED OUTCOMES
Tumors will mostly form and grow in the pylorus and antrum of stomach (Figure 2B), sometimes in the corpus. Furthermore, a swelling/hyperplasia of the epithelia can be found in the non-tumor area. Due to the heterogeneity of the size and number of tumors in the animals, we usually use more than 10 mice. In case of drug treatment or use of genetically modified mice, the time for gastric tumor development may change.

The protocol of MNU with high concentration (short treatment time) is commonly used; low concentration may require long time of drug treatment to induce gastric tumor formation (Yamachika et al., 1998). We summarized the incidence of tumors in the stomach and other organs in various strains (Table 1).

QUANTIFICATION AND STATISTICAL ANALYSIS
The numbers of tumors in the stomach are counted visually. For the volume, the length, width, and height of each tumor/polyp are measured by vernier caliper, multiplying length by width and height to get the tumor volume.

LIMITATIONS
MNU-induced murine tumor model can be used to study the initiation and development of gastric tumors. It mimics human gastric cancer in several ways: somatic DNA mutations, microbial infection, and similarity between MNU and diet-contained nitrate. Furthermore, MNU can induce hyperplasia (polyps) and adenoma, similar to human gastric tumors. However, there are limitations for this model. The risk factors are much complex in humans and MNU only mimics diet-contained nitrate. Due to the long time needed for the development of tumors, mice may die prematurely especially in mice with genetic modifications. Moreover, there is no obvious sign to assess tumor formation before sacrificing the mice.

TROUBLESHOOTING
Problem
The amount of MNU powder is too little to weigh (step 2).

Potential solution
We recommend the smallest measuring scoop or hand-made small scoop using a plastic straw, which, together with MNU, can be directly dropped into the light-shielded bottle.
Problem
Mouse death during the treatment (step 10).

Potential solution
MNU may affect other organs and tissues, such as lung, kidney and liver, causing injury and/or carcinogenesis. The incidence of lung, kidney and liver hyperplasia is 11, 5 and 18 percent respectively (detailed in Table 1). Low concentrations may reduce mouse mortality. For example, the concentration of MNU can be 120 ppm and the number of cycle is still 5.

Problem
Tumor in the duodenum (step 15).

Potential solution
The main region of gastric tumor initiation and development is antrum and corpus. However, sometimes the tumors growing in the pylorus may extend into the duodenum, with an incidence of 6%. When harvesting the tissues, you need carefully remove the gut and keep a part of duodenum with tumors, open it and take photos.

Problem
No tumor observed in the stomach in some animals (step 15).

Potential solution
In MNU-induced models, the incidence of tumors exhibits some variability. However, the incidence of dysplasia in wild-type mice is 60%–90%, which may increase to 100% with H. pylori infection (Sethi et al., 2020; Tomita et al., 2011). Alternatively, some small polyps can be observed and measured under dissecting microscope, which are also suitable for histopathological analysis (Figure 2D).

Problem
Failure of perfusion (step 13).

Potential solution
At the beginning, you should immediately and carefully open the thoracic cavity after anesthesia to avoid blood coagulation or hemorrhage. You need correctly insert the syringe needle into the left ventricle and break the right auricle to establish the perfusion loop. Moreover, the perfusion solution should be injected at a slow and constant speed to prevent vessel bursting.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Baojie Li (libj@sjtu.edu.cn)

Materials availability
This study did not generate new unique reagents.

Data and code availability
This study did not generate/analyze datasets.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100814.

ACKNOWLEDGMENTS
The work as supported by the National Key R&D Program of China (2018YFA0800803 and 2017YFA0103602) and the National Natural Science Foundation of China (81520108012 and 91749201),
AUTHOR CONTRIBUTIONS

B.L. designed the research; K.L. and A.W. wrote the original draft; B.L. and H.L. reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Faustino-Rocha, A.I., Ferreira, R., Oliveira, P.A., Gama, A., and Ginja, M. (2015). N-Methyl-N-nitrosourea as a mammary carcinogenic agent. Tumour Biol. 36, 9095–9117.

Lee, S.H., Park, J.W., Go, D.M., Kim, H.K., Kwon, H.J., Han, S.U., and Kim, D.Y. (2015). Ablation of osteopontin suppresses N-methyl-N-nitrosourea and Helicobacter pylori-induced gastric cancer development in mice. Carcinogenesis 36, 1550–1560.

Li, K., Wu, H., Wang, A., Charron, J., Mishina, Y., Habib, S.L., Liu, H., and Li, B. (2021). mTOR signaling regulates gastric epithelial progenitor homeostasis and gastric tumorigenesis via MEK1-ERKs and BMP-Smad1 pathways. Cell Rep. 35, 109069.

Masui, T., Tezuka, N., Nakanishi, H., Inada, K., Miyashita, N., and Tatematsu, M. (1997). Induction of invasive squamous cell carcinomas in the forestomach of (C3H x MSM)F1, MSM, and C3H mice by N-methyl-N-nitrosourea and mutational analysis of the H-ras and p53 genes. Cancer Lett. 111, 97–104.

Ogawa, K., Murasaki, T., Sugiuira, S., Nakanishi, M., and Shirai, T. (2013). Organ differences in the impact of p27(kip1) deficiency on carcinogenesis induced by N-methyl-N-nitrosourea. J. Appl. Toxicol. 33, 471–479. Pazos, P., Lanari, C., Meiss, R., Charreau, E.H., and Pasqualini, C.D. (1992). Mammary carcinogenesis induced by N-methyl-N-nitrosourea (MNJ) and medroxyprogesterone acetate (MPA) in BALB/c mice. Breast Cancer Res. Treat. 20, 133–138.

Qin, X., Shibata, D., and Gerson, S.L. (2000). Heterozygous DNA mismatch repair gene PMS2-knockout mice are susceptible to intestinal tumor induction with N-methyl-N-nitrosourea. Carcinogenesis 21, 833–838.

Sethi, N.S., Kikuchi, O., Duronio, G.N., Stachler, M.D., Mcfarland, J.M., Ferrer-Luna, R., Zhang, Y., Bao, C., Bronson, R., Patil, D., et al. (2020). Early TP53 alterations engage environmental exposures to promote gastric premalignancy in an integrative mouse model. Nat. Genet. 52, 219–230.

Soloway, M.S., Nisenkom, I., and McCallum, L. (1983). Urothelial susceptibility to tumor cell implantation: comparison of cauterization with N-methyl-N-nitrosourea. Urology 21, 159–161.

Tatematsu, M., Yamamoto, M., Iwata, H., Fukami, H., Yuasa, H., Tezuka, N., Masui, T., and Nakanishi, H. (1993). Induction of glandular stomach cancers in C3H mice treated with N-methyl-N-nitrosourea in the drinking water. Jpn. J. Cancer Res. 84, 1258–1264.

Tomita, H., Takashi, S., Menhenoti, T.P., Yang, X., Shibata, W., Jin, G., Betz, K.S., Kawakami, K., Minamoto, T., Tomasetto, C., et al. (2011). Inhibition of gastric carcinogenesis by the hormone gastrin is mediated by suppression of TFF1 epigenetic silencing. Gastroenterology 140, 879–891.

Westcott, P.M., Halliwell, K.D., To, M.D., Rashid, M., Rust, A.G., Keane, T.M., Delrosso, R., Jen, K.Y., Gurley, K.E., Kemp, C.J., et al. (2015). The mutational landscapes of genetic and chemical models of Kras-driven lung cancer. Nature 517, 489–492.

Yamachika, T., Nakamichi, H., Inada, K., Tsukamoto, T., Shimizu, N., Kobayashi, K., Fukushima, S., and Tatematsu, M. (1998). N-methyl-N-nitrosourea concentration-dependent, rather than total intake-dependent, induction of adenocarcinomas in the glandular stomach of BALB/c mice. Jpn. J. Cancer Res. 89, 385–391.

Yamamoto, M., Tsukamoto, T., Sakai, H., Shirai, N., Ohgaki, H., Furihata, C., Donehower, L.A., Yoshida, K., and Tatematsu, M. (2000). p53 knockout mice (-/-) are more susceptible than (+/-) or (+/+) mice to N-methyl-N-nitrosourea stomach carcinogenesis. Carcinogenesis 21, 1891–1897.

Yoshizawa, N., Yamaguchi, H., Yamamoto, M., Shimizu, N., Furihata, C., Tatematsu, M., Seto, Y., and Kamimishi, M. (2009). Gastric carcinogenesis by N-Methyl-N-nitrosourea is enhanced in db/db diabetic mice. Cancer Sci. 100, 1180–1185.