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Original Article

A deficiency in cathelicidin reduces lung tumor growth in NNK/NTHi-induced A/J mice

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Abstract: Cathelicidin is an antimicrobial peptide that plays an essential role in cell proliferation, angiogenesis, and also has been indicated in tumor promotion. However, it is unclear how cathelicidin causes tumor growth, and the pathogenic mechanisms based on gain or loss of function have not been proposed. Here, a cathelicidin related antimicrobial peptide (CRAMP) knockout mouse was generated using an A/J background (A/J-CRAMP$^{-/-}$ mice), and lung carcinoma growth was induced using 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and Non-typeable Haemophilus influenzae (NTHi). Compared with A/J mice, A/J-CRAMP$^{-/-}$ mice were found to have a lower tumor burden and longer survival times, with a significant reduction in both PCNA and Ki-67 positive cells. However, there was no difference between the number of apoptotic lung-cancer cells between the A/J and A/J-CRAMP$^{-/-}$ mice. This indicated cathelicidin might be a tumor growth factor for lung cancer, which was associated for proliferation of tumor cells. In the future, this animal model will be useful to study the distinct role of cathelicidin in induced-lung cancer development.

Keywords: Cathelicidin, lung cancer, NNK/NTHi, A/J mice

Introduction

Cathelicidin is an antimicrobial peptide with direct antibacterial activity and various immune functions [1]. The human gene *CAMP* codes for the only known cathelicidin protein in human cells, known as human cationic antimicrobial peptide-18 (hCAP18) [2]. The LL-37 peptide is the C-terminal peptide of hCAP18, released by cleavage of protease3 after hCAP18 activation [3]. In mice, the gene *Cnlp* is homologous to the human gene *CAMP,* and codes for the cathelicidin related antimicrobial peptide (CRAMP). CRAMP is the only known mouse cathelicidin peptide having similar α-helical structure, spectra of antimicrobial function, and analogous tissue distribution to LL-37 [4, 5]. Recently, other various biological functions of cathelicidin have been identified, such as wound healing, cell proliferation, angiogenesis, and regulation of cell migration [6, 7]. Emerging evidence implicates that cathelicidin is even involved in the promotion of tumor growth [3, 8]. However, the mechanism of cathelicidin in tumor promotion is poorly understood.

To explore the mechanism of cathelicidin in tumor development, a suitable mouse model with a genetic modification of CRAMP is necessary. In 2001, Nizet *et al.* established a *Cnlp-null* mouse model for studying the role of CRAMP in protection against skin infection by bacteria [5]. CRAMP knockdown mice have also been used to study prostate cancer associated with decreased cellular proliferation and invasion [9]. In a previous study, CRAMP$^{-/-}$ mice were used to study lung cancer metastasis by intravenously injecting Lewis lung carcinoma (LLC) cells into the tail vein, and evaluating the function of CRAMP in the tumor microenvironment [10]. However, this work was performed using mice with a C57BL/6J background, which is a lung tumor resistant mouse strain, and are, therefore, not an optimal model to study

induced lung cancer. Compared with the C57BL/6J strain, A/J mice exhibit high susceptibility to spontaneous and chemically induced lung tumors [11], which makes them as an optimal model for lung carcinogenesis research.

The purpose of this study is to establish a novel CRAMP deficient mouse model with an A/J background (A/J-CRAMP$^{-/-}$). This model can then be used to study the role of the antimicrobial peptide cathelicidin on induced lung tumor formation, which may prepare for further mechanistic research.

Materials and methods

Animals

CRAMP$^{-/-}$ mice in a C57BL/6J background were described earlier, and kindly supplied by Prof. Robert Bals (Saarland University Hospital, Germany) [12]. A/J mice were obtained from Shanghai Experimental Animal Slack limited liability company, qualified SCXK (Shanghai) 2012-0002. Mice were maintained in a pathogen-free, Central Animal Facility of the Tongji Hospital of Tongji University. This study was carried out in strict accordance with the recommendations found in the Guidelines for the Care and Use of Laboratory Animals of the National Institute of Health. All animal experiments were approved by the Tongji Hospital of Tongji University Ethics Committee on the Care and Use of Animals. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Briefly, to generate a constitutive knockout of CRAMP mice in an A/J background, the C57BL/6J Cramp$^{-/-}$ mice were backcrossed with A/J animals for 10 generations. The steps are as follows: C57BL/6J Cramp$^{-/-}$ mice (black) were crossed with A/J mice (white) to obtain a heterozygous genotype of CRAMP mice, designated as the F1 generation. White, male, F1 mice were selected and backcrossed with A/J mice 10 times to purify the genetic background. Mice obtained after 10 generations were intercrossed to obtain homologous mice with *Cnlp*-null genes. Polymerase chain reaction (PCR) was used to detect wild-type and *Cnlp*-null genes to confirm homozygosity of the CRAMP knockout mice.

Genotype analysis

Genotypes of mice bearing the A/J wild-type, A/J-CRAMP$^{+/-}$ and A/J-CRAMP$^{-/-}$ were determined by PCR analysis. DNA was extracted from the mouse tail, using a nucleic acid purification kit (Cat.no.RR820Q, Takara, JPN). The primers used were 14087 (5'-CCAGGACGAGGATCCAGATA-3'), 14088 (5'-CCCATACACTGCTTCACCAC-3') and oIMR7996 (5'-CTTCCATTTGTCACGTCCTG-3'), obtained from Jackson Laboratory. PCR was performed using an ABI-7300 (Thermo Scientific, USA) with the following conditions: 94°C for 2 min, 10 cycles for (94°C, 20 sec; 65°C, 15 sec; 68°C, 10 sec), 28 cycles for (94°C, 15 sec; 60°C, 15 sec; 72°C, 10 sec), 72°C for 2 min, followed by a 10°C hold. After PCR amplification, an agarose gel was used to determine the size of the resulting fragments. A mutant allele generated a 474 base pair fragment, where as a wild-type allele generated a 269 base pair fragment.

RNA extraction and real time PCR

RNA was extracted from the lung of mice using Trizol (Cat.no.15596026, Invitrogen, USA) reagent according to the manufacturer's instructions. Single strand cDNA was synthesized from 400 ng of total RNA using an RNA reverse transcriptase kit (Cat.no.RR036A, Takara, JPN). The mRNA level of CRAMP was detected using primers CRAMP-F (5'-CTTCAACCAGCAGTCCCTAGACA-3') and CRAMP-R (5'-TCCAGGTCCAGGAGACGGTA-3'). The β-actin gene was detected by primers β-actin-F (5'-CAGATGTGGATCAGCAAGCAGG-3') and β-actin-R (5'-TTGTCAAAGAAAGGGTGTAAAACG-3').

Preparation of total protein extract and western blotting

Total protein in the lung tissue was extracted using RIPA lysis buffer (Cat.no.9806, CST, USA) on ice. The tissue was centrifuged at 4°C and 12000 r/min for 20 min, then the supernatant was placed in a fresh tube. Total protein was quantified using a BCA assay (Cat.no.p0010, Beyotime Biotechnology, CHN). The protein samples (40 μg) were then subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using β-mercap-toethanol and bromophenol blue as buffers. The separated protein samples were transferred

onto a nitrocellulose filter membrane (Cat.No.-10401196, whatman, USA). The membranes were soaked for 1 h at room temperature in a blocking buffer (1 × TBST with 5% w/v nonfat dry milk). These were then incubated with antibody CRAMP (1:100 dilution, Cat.no.sc-166-055, Santa Cruz, USA), and mouse antibody β-actin (1:1000 dilution, Cat.no.sc-47778, Santa Cruz, USA) overnight at 4°C. After overnight incubation, the membranes were washed 3 times with blocking buffer, and blotted with secondary horseradish peroxidase (HRP)-conjugated antibody (1:2000 dilution, Cat.no. p0161, Dako, DEN) at room temperature for 1 h. Under these same test conditions, the level of β-actin expression was used as a control standard. An ECL western blotting detection system (Cat.No.7003, CST, USA) was used to measure the protein bands.

Histologic and immunohistochemical analyses

The lung cancer tissues were infused with 10% formaldehyde (Cat.no.E672001, sangon, CHN), and processed for serial paraffin sections (4 μm). One section was stained with hematoxylin and eosin (H&E). Other sections were prepared for immunohistochemical analysis. These sections were deparaffinized, and sodium citrate (Cat.no.22F00120, Dingguo, CHN) was used for antigen retrieval at 98°C for 15 min during hot water treatment. Endogenous peroxidase activity was blocked using 3% (V/V) H_2O_2 in PBS for 12 min, to prevent nonspecific binding. For the primary antibody labeling, the slides were incubated with rabbit anti-Ki-67 (1:100, Cat.no.ab16667, Abcam, UK) and anti-PCNA (1:100, Cat.no.13110, CST, USA) at 4°C overnight. Tissue sections were balanced at room temperature for 1 hour, and then washed 3 times for 5 min each in PBS the second day. Finally, slides were soaked with a streptavidin/peroxidase complex (Cat.no.k500705, Gene, CHN). Brown staining in the nucleus was considered an indicator of protein expression, and counted using a microscope (Olympus, JPN) at a magnification of × 200 and × 400. For this purpose, five fields per slide were randomly selected by the viewer for evaluation.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

Mice were treated with NNK/NTHi. The lungs were removed, fixed, and paraffin embedded after 4 months. Apoptosis in the lung sections was examined following TUNEL staining (Cat. no.G7360; Promega, USA). The 4 μm paraffin-embedded sections were deparaffinized with xylene, and the slides washed using decreasing concentrations of ethanol (100%, 100%, 95%, 75%) for 5 min each time. Next, sections were washed 3 times for 3 min each in PBS, then TdT reaction mixture was added and the sections incubated for 60 min at 37°C in a humidified chamber. After incubation, sections were washed 3 times in PBS for 3 min each, and then immersed in 2 × SSC for 15 min. Slides were then washed 3 times for 3 min each in PBS, and incubated with Streptavidin HRP (diluted 1:500 in PBS) for 30 min at room temperature. Finally, sections were washed 3 times for 3 min each in PBS and incubated with DAB substrate until a light brown background appeared. TUNEL-positive particle were counted using a microscope (Olympus, JPN) at a magnification of × 200 and × 400.

Statistical analysis

Data is expressed as the Mean ± S.E.M. The data was analyzed using a two-tailed, unpaired Student's t-test, or Kaplan-Meier survival analysis. A *P*-value of < 0.05 was considered statistically significant.

Results

Generation of A/J mice lacking CRAMP

It has been reported that 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) stimulation with Non-typeable Haemophilus influenzae (NTHi) exposure can accelerate induced lung cancer promotion and progression in A/J mice [13]. In order to study the role of the CRAMP gene in induced lung cancer, eight-week-old A/J mice (white) were mated with C57BL/6J-CRAMP$^{-/-}$ mice (black) to successfully breed offspring. Results showed genotypes of the first-generation mice (F1) were all CRAMP$^{-/-}$ heterozygotes with 75% black pups and 25% white pups. According to Mendel's law, F1 white mice receive 50% of their genetic information from the A/J background. To obtain mice with a pure A/J background, homozygous transgenic mice and white heterozygous siblings were backcrossed with A/J mice for ten generation (F10), resulting in 99.9% A/J genetic information. Then, the F10 mice were intercrossed,

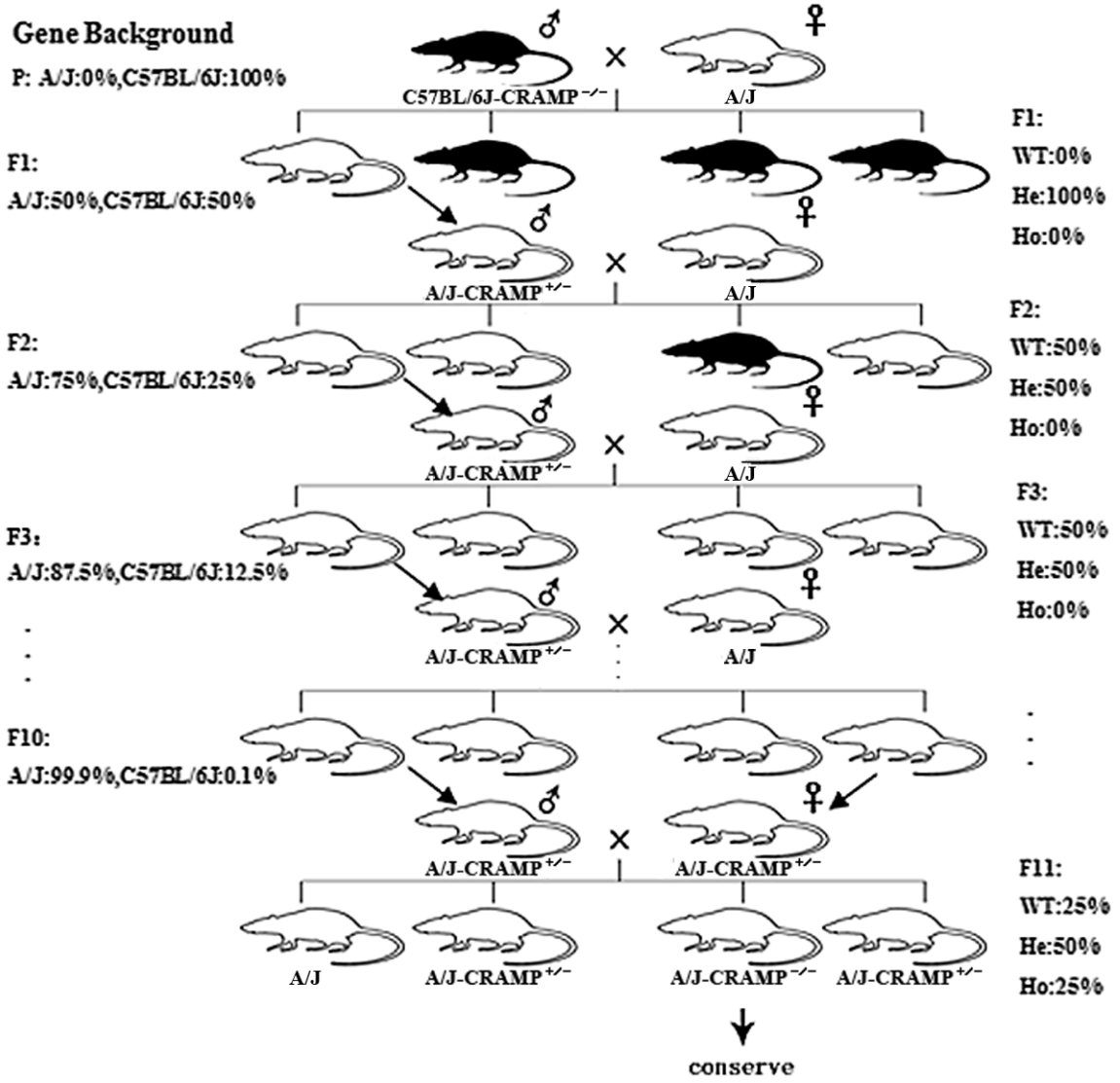

Figure 1. Generation of a congenic knockout strain using a traditional backcrossing breeding strategy. The CRAMP knockout mouse (P: parental) was originally generated from using a C57BL/6J strain embryonic stem cell line. An initial cross between the parental to an A/J mouse is shown in the first line. Male carriers of the knockout allele were identified (CRAMP KO), and the F1 offspring were used as the parents of the second backcross. F1 carriers are genetically 50% of the original background strain (C57BL/6J), and 50% of the recipient strain (A/J). F1*A/J crosses were used to generate F2 offspring, which dilutes the original genetic background by another 50%. The backcross matings were successively preformed until the 10th backcross, which resulted in mice that should be genetically 99.9% A/J strain. At each mating, the offspring are genotyped for carrying the knockout allele. The gradual darkening of the mice represented the gradual increase in A/J content.

generating three genotypes, CRAMP$^{-/-}$ homozygote, CRAMP$^{+/-}$ heterozygote, and wild-type, all of them with a white phenotype (**Figure 1**). The homozygous A/J-CRAMP$^{-/-}$ mice were used to maintain the line in a homozygous state. Genotyping PCR was used to identify wild-type, heterozygous, and homozygous transgenic mice (**Figure 2**).

RT-PCR was used to detect CRAMP mRNA expression in the three mouse genotypes CRAMP$^{-/-}$ homozygote, CRAMP$^{+/-}$ heterozygote, and wild-type. The results revealed there was almost 50% of CRAMP mRNA expression in A/J-CRAMP$^{+/-}$ mice compared with wild-type mice ($P < 0.05$), while nearly no CRAMP mRNA expression was detected in the CRAMP knock-

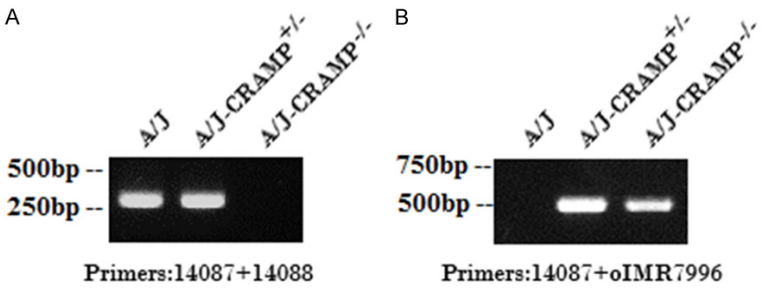

Figure 2. PCR genotype results of tail DNA amplified with CRAMP and A/J specific primers. Primer pair 14087/14088 produced a fragment 269 base pair in size from A/J and A/J-CRAMP$^{+/-}$, and no amplification for A/J-CRAMP$^{-/-}$ (A). Primer pair 14087/oIMR7996 produces a fragments 474 base pair in size from A/J-CRAMP$^{+/-}$ and A/J-CRAMP$^{-/-}$, and no amplification for A/J (B).

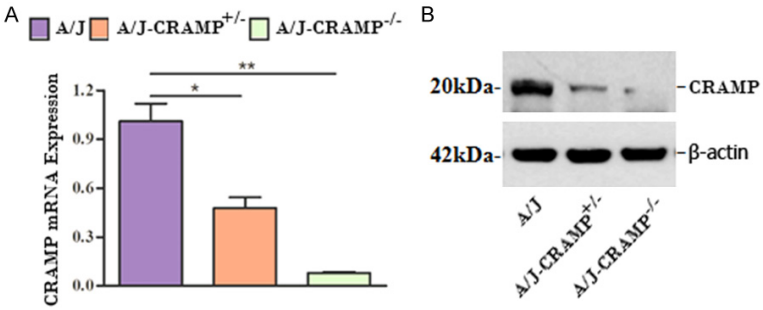

Figure 3. The expression of CRAMP gene in molecular and protein levels. RNA and total protein from A/J, A/J-CRAMP$^{+/-}$ and A/J-CRAMP$^{-/-}$ mouse were isolated from F10. The expression of CRAMP mRNA in three genetic mouse was measured by real-time PCR (A). The expression of CRAMP protein was analyzed by western blot (B). The results were expressed as the mean ± SEM, $*P < 0.05$, $**P < 0.01$.

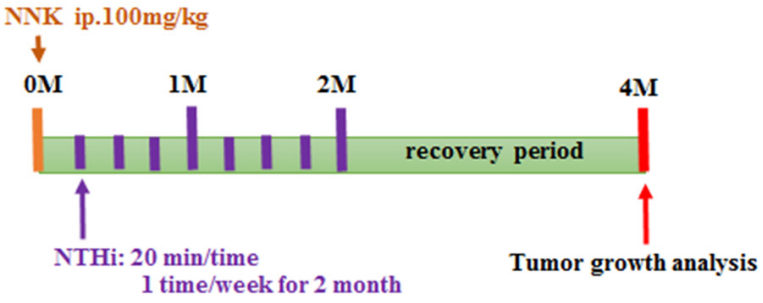

Figure 4. Experimental protocol of NTHi/NNK-induced lung cancer in A/J mouse model. Eight week-old A/J and A/J-CRAMP$^{-/-}$ mice received abdominal injections of NNK (100 mg/kg); at day 7, mice were NTHi (2.5 mg/ml) exposed for 1 time a week for 2 months. Tumor growth was analyzed 4 months later.

out mice ($P < 0.01$) (**Figure 3A**). Western blot analysis was used to confirme that there was no CRAMP protein detected in A/J-CRAMP$^{-/-}$ mice, even though CRAMP protein was present in both wild-type and A/J-CRAMP$^{+/-}$ mice (**Figure 3B**). Taken together, these results demonstrated that a CRAMP knockout mouse model with an A/J background was successfully generated.

Effect of CRAMP on NNK/NTHi-induced lung tumor progression in A/J mice

It has been previously demonstrated that the host defense peptide LL-37/hCAP-18 functions as a growth factor for lung cancer cells [14]. Previous results showed that cathelicidin CRAMP promoted metastatic lung tumor growth in C57BL/6J mice [10]. To investigate the role of CRAMP in chemically induced lung carcinoma, NNK was injected into CRAMP-knockout mice (A/J-CRAMP$^{-/-}$) and littermate control mice (A/J), followed by NTHi exposure (**Figure 4**). Analysis of lung and histological appearance found that the tumor load of A/J mice was much higher than A/J-CRAMP$^{-/-}$ mice, and A/J-CRAMP$^{-/-}$ showed a significant reduction of lung weight, number of lung nodules, and lung tumor max diameter (**Figure 5**) ($P < 0.001$). NNK/NTHi stimulation resulted in tumor growth in A/J mice, whereas A/J-CRAMP$^{-/-}$ were protected from NNK/NTHi-induced tumor growth (**Figure 5**). In the survival analysis, A/J-CRAMP$^{-/-}$ exhibited longer survival times than A/J mice with a median survival time of 190 d vs. 136.5 d (**Figure 6**).

Effect of CRAMP on proliferation and apoptosis of NNK/NTHi-induced A/J mice

The results above showed that the lung weight, tumor nodules, and tumor max diameter in

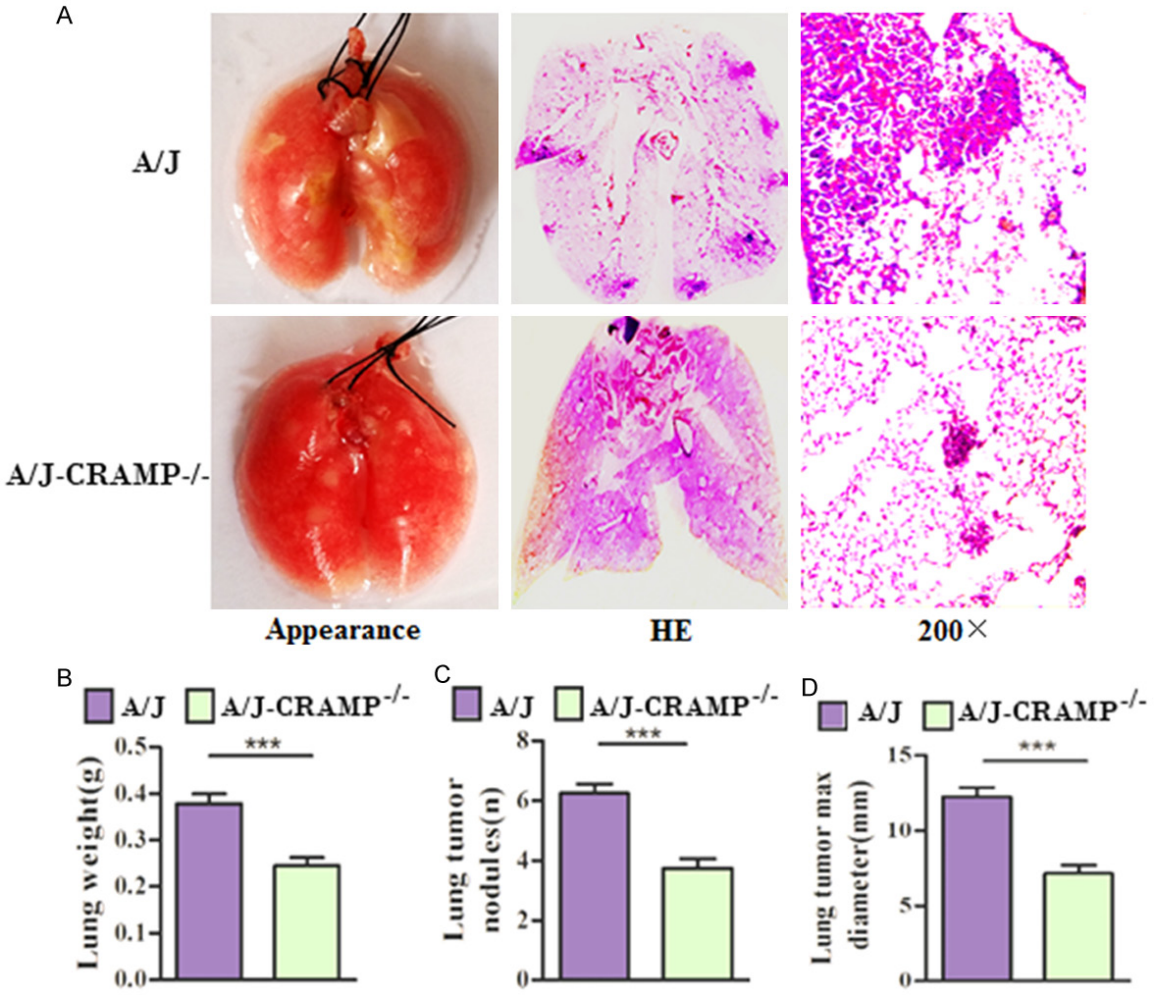

Figure 5. Lung and histological appearance of cancer in NNK + NTHi exposed mice. Lung appearance (left) and histology (H&E stain; right) in A/J and A/J-CRAMP$^{-/-}$ mice 4 months after initiation of the NNK + NTHi protocol. (A) NNK (100 mg/kg) were the abdominally injected into A/J and A/J-CRAMP$^{-/-}$ mice; at day 7, mice were NTHi (2.5 mg/ml) exposed for 1 time a week for 2 month; at 4 months, the lungs were removed. Lung weight (B), the numbers of tumor nodules (C) and lung tumor max diameter (D) detectable on the lung surface were determined (n = 20, significant difference, ***$P < 0.001$).

A/J mice without CRAMP were significantly decreased, and the survival time was significantly prolonged. This indicates CRAMP plays an important role in lung tumor development, however, it is not clear whether it inhibits the proliferation of tumor cells or promotes the apoptosis of tumor cells. Therefore, immunohistochemical staining for PCNA, Ki-67, and TUNEL was used to detect the effects of CRAMP on NNK/NTHi-induced lung tumors. The effect of CRAMP on lung adenocarcinoma cell proliferation was assessed through labeling of PCNA and Ki-67 positive cells, which were reduced significantly in tumors from CRAMP knockout mice compared with A/J mice ($P < 0.001$) (**Figure 7**). However, using microscopy it was determined that the typical features of apoptotic cell particles were not observed in lung cancer from A/J and A/J-CRAMP$^{-/-}$ mice (**Figure 8**).

Discussion

Lung cancer is one of the deadliest cancer forms, and accounts for more than one million deaths per year worldwide [15]. This high death rate is mainly due to the late diagnosis, poor prognosis and lack of effective treatment. Therefore, mechanism study of lung cancer will provide valuable evidences for further preventive strategies and treatments. Emerging evidence indicates that cathelicidin can promote

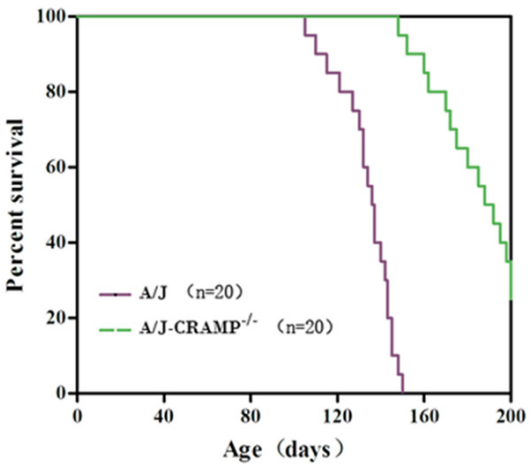

Figure 6. Kaplan-Meier Survival curves of A/J mice and A/J-CRAMP$^{-/-}$ mice were the abdominally injected with NNK (100 mg/kg) exposed to NTHi (2.5 mg/ml) (Log-rank test for statistical analysis; n = 20). All results were confirmed in at least one additional independent experiment.

lung tumor formation, although mechanisms are yet not elucidated [14]. Therefore, in the current work, an induced lung cancer model in A/J mice with CRAMP deficiency was generated and used to explore the roles of cathelicidin in lung cancer.

CRAMP$^{-/-}$ mice model was originally established in 2001 by Nizet et al to study skin bacterial infection. The CRAMP$^{-/-}$ genotype was generated by replacing exons 3 and 4 of the *Cnlp* gene, which encode the entire mature domain of CRAMP with PGK-neo in C57BL/6J mice [5]. This C57BL/6J-CRAMP$^{-/-}$ mice model was also used to study metastasis lung cancer in previous study by intravenously injecting LLC cells into the tail vein [10]. Metastasis cancer model has the characteristics of establishment model in short cycle, high success rate, and good repeatability. However, as the short survival time of metastasis cancer mice, there are certain limitations on further mechanism research [16]. Meanwhile, it has been demonstrated that C57BL/6J mice are highly insensitive to induced lung cancer. Whereas, A/J strain exhibit high susceptibility to spontaneous and/or chemically induced lung tumors [11]. Lung tumors in A/J mice display many morphological, histopathological, and molecular similarities to human pulmonary adenocarcinomas, which makes them a relevant model for lung carcinogenesis research [17, 18]. Therefore, in present study, we transferred the CRAMP$^{-/-}$ gene from the C57BL/6J background to the A/J background via 10 generations backcrossing to obtain a purified A/J background, CRAMP knockout mice. CRAMP was absent in the A/J-Cramp$^{-/-}$ mouse by verification of both PCR and western blot analysis (**Figures 2**, **3**). These results indicate the successfully establishment of A/J-Cramp$^{-/-}$ mouse model.

NNK is one of the most potent carcinogens from cigarette smoke for humans and laboratory animals, and causes tobacco-associated lung cancer by inducing chronic airway inflammation [19]. NTHi is the most common bacterial colonizer of airways in COPD patients [20, 21], and shows to promote lung tumorigenesis in an oncogenic K-ras induced mouse model [13]. It has been reported that chronic airway inflammation caused by tobacco smoke and microbial infection promote lung carcinogenesis [22, 23], and the risk of lung cancer is positively related to the severity and duration of inflammation [24]. Therefore, we used NTHi and NNK exposure together to promote the formation of lung cancer in an A/J mouse model with and without CRAMP activity in present study, which mimics the typical course of lung cancer caused by a combination of bacterial infection and carcinogen exposure. The results showed NNK/NTHi exposure resulted in a significance enhanced tumor growth in wild type A/J mice, and the median survival time was 136.5 d (**Figures 5**, **6**). Immunohistochemical staining for PCNA and Ki-67 demonstrated a large amount of positive cells, which supports NNK/NTHi exposure promoted tumor cells proliferation (**Figure 7**).

Cathelicidin was initially known as its significant antibacterial and bactericidal effects [25]. Subsequent studies found that cathelicidin is also overexpressed in a variety of tumor cells, such as ovarian cancer, colorectal cancer, and prostate cancer [26-28]. The upregulation of LL-37 and production of the cathelicidin protein have been observed in human lung cancer, and administration of synthetic and biologically active LL-37 peptide, or transgenic expression of LL-37 in tumor cells, promotes lung tumor cell proliferation [14]. Multiple mechanisms might be involved in cathelicidin activation of tumor growth: (1) Cathelicidin maintains the inflammatory state in the tumor microenvironment, recruits inflammatory cells to promote tumor proliferation,

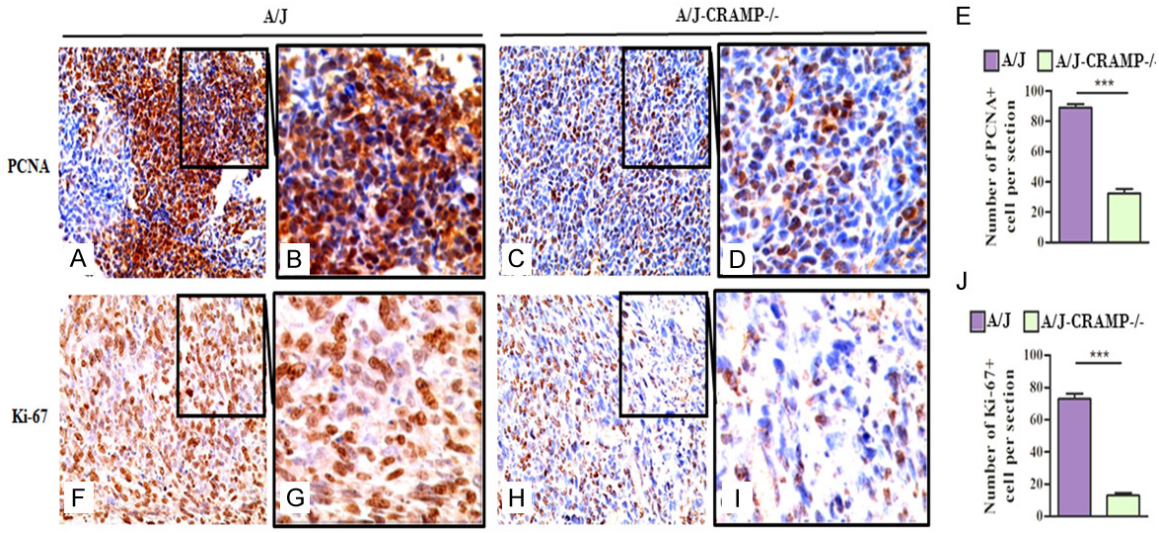

Figure 7. Immunohistochemical staining for PCNA (A-D) and Ki-67 (F and I) expression in lung cancer from A/J and A/J-CRAMP$^{-/-}$ mice. Immunohistochemical analysis of lung tissue following 4 months of NNK treatment, section showed strong PCNA (A: 200 ×, B: 400 ×) and Ki-67 (F: 200 ×, G: 400 ×) staning of tumor cells in A/J mice group, weak PCNA (C: 200 ×, D: 400 ×) and Ki-67 (H: 200 ×, I: 400 ×) staning in A/J-CRAMP$^{-/-}$ mice group. The average number of PCNA+ and Ki-67+ cell per section were shown as the mean ± SEM (n = 20). The average number of PCNA+ (E) and Ki-67+ (J) cell in A/J-CRAMP$^{-/-}$ group were significantly decreased as compared with A/J mice group.***$P < 0.001$, t-test.

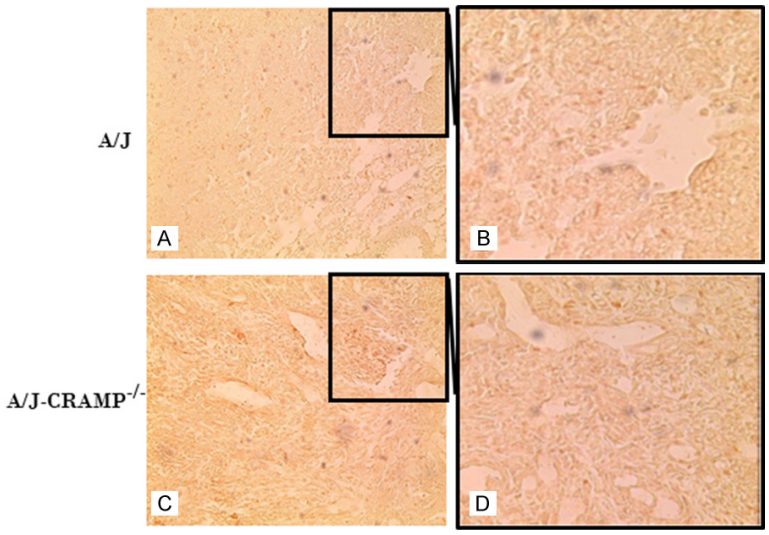

Figure 8. Tissue cell apoptosis in lung cancer from A/J and A/J-CRAMP$^{-/-}$ mice. Mice were treated by NNK + NTHi treatment, following 4 months the lungs were removed, fixed, and paraffin embedded. Paraffin-embedded tumor lung sections were examined by TUNEL-histochemistry assay. Representative microphotographs of TUNEL staining of A/J groud (A: 200 ×, B: 400 ×) and A/J-CRAMP$^{-/-}$ groud (C: 200 ×, D: 400 ×) . Each microscopic view positive particle data is shown as the mean ± SEM (n = 20), Microscopy shown typical feature of apoptotic cell particles were not observed in lung cancer from A/J and A/J-CRAMP$^{-/-}$ mice (data not shown).

invasion, adhesion, and angiogenesis [10]; (2) Cathelicidin regulates tumors progression by activating TLR2, vitamin D3, and Wnt/β-catenin signaling pathways [8, 14]; (3) Cathelicidin regulates angiogenesis and promotes tumor cell metastasis [29]. Therefore, as a tumor growth factor, the pathomechanisms study of cathelicidin in lung cancer has the potential prospects for further preventive strategies and treatments. In our current work, we successfully established CRAMP$^{-/-}$ mouse model, and the results showed there were lower tumor burdens and longer survival times in CRAMP$^{-/-}$ mice after NNK/NTHi exposure (**Figures 5**, **6**). This support the previous theoretical basis that cathelicidin could promote tumor growth. We also found PCNA and Ki-67 positive cells were significant reduced in tumors of CRAMP deleted mice. However, there was no difference in the number of apoptotic lung cancer cells between A/J and A/J-CRAMP$^{-/-}$ mice (**Figures 7**, **8**). These data suggest that cathelicidin could promote tumor growth by

inducing tumor cell proliferation, rather than inhibiting cell apoptosis. The mechanisms would be our further exploration.

In summary, we successfully generated a mouse model containing a deletion of CRAMP in an A/J background (A/J-CRAMP$^{-/-}$). This novel mouse model can be used to explore the role of CRAMP in lung cancer development, and provide new tool to study the molecular mechanisms that may contribute to the formation of lung cancer.

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Disclosure of conflict of interest

None.

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