Title: Deletion of Nardilysin Prevents the Development of Steatohepatitis and Liver Fibrotic Changes

Author(s): Ishizu, Shoko

Citation: Kyoto University (京都大学)

Issue Date: 2015-01-23

URL: https://doi.org/10.14989/doctor.k18682

Copyright: © 2014 Ishizu-Higashi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Type: Thesis or Dissertation

Textversion: ETD Kyoto University
Deletion of Nardilysin Prevents the Development of Steatohepatitis and Liver Fibrotic Changes

Shoko Ishizu-Higashi1, Hiroshi Seno1, Eiichiro Nishi2, Yoshhide Matsumoto1, Kozo Ikuta1, Motoyuki Tsuda1, Yoshito Kimura1, Yutaka Takada1, Yuto Kimura1, Yuki Nakanishi1, Keitaro Kanda1, Hideyuki Komekado1, Tsutomu Chiba1

1 Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Kyoto, Japan, 2 Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

Abstract

Nonalcoholic steatohepatitis (NASH) is an inflammatory form of nonalcoholic fatty liver disease that progresses to liver cirrhosis. It is still unknown how only limited patients with fatty liver develop NASH. Tumor necrosis factor (TNF)-α is one of the key molecules in initiating the vicious circle of inflammations. Nardilysin (N-arginine dibasic convertase; Nrd1), a zinc metalloendopeptidase of the M16 family, enhances ectodomain shedding of TNF-α, resulting in the activation of inflammatory responses. In this study, we aimed to examine the role of Nrd1 in the development of NASH. Nrd1−/− and Nrd1+/−/− mice were fed a control choline-supplemented amino acid-defined (CSAA) diet or a choline-deficient amino acid-defined (CDA) diet. Fatty deposits were accumulated in the livers of both Nrd1−/− and Nrd1+/−/− mice by the administration of the CSAA or CDAA diets, although the amount of liver triglyceride in Nrd1−/− mice was lower than that in Nrd1+/−/− mice. Serum alanine aminotransferase levels were increased in Nrd1+/−/− mice but not in Nrd1−/− mice fed the CDA diet. mRNA expression of inflammatory cytokines were decreased in Nrd1+/−/− mice but in Nrd1−/− mice fed the CDA diet. While TNF-α protein was detected in both Nrd1+/−/− and Nrd1−/− mice, secretion of TNF-α in Nrd1−/− mice was significantly less than that in Nrd1+/−/− mice, indicating the decreased TNF-α shedding in Nrd1−/− mouse liver. Notably, fibrotic changes of the liver, accompanied by the increase of fibrogenic markers, were observed in Nrd1+/−/− mice but not in Nrd1−/− mice fed the CDA diet. Similar to the CDAA diet, fibrotic changes were not observed in Nrd1−/− mice fed a high-fat diet. Thus, deletion of nardilysin prevents the development of diet-induced steatohepatitis and liver fibrogenesis. Nardilysin could be an attractive target for anti-inflammatory therapy against NASH.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a condition in which excess fat accumulates in the hepatocytes of patients without a history of alcohol abuse [1]. NAFLD is a hepatic manifestation of metabolic syndromes, such as obesity, type-II diabetes mellitus, and hyperlipidemia. Its prevalence is increasing particularly in the developed countries [1,2]. Nonalcoholic steatohepatitis (NASH) is a severe form of NAFLD, in which liver inflammation is observed and which progresses to liver fibrosis. A part of NAFLD patients develops NASH that leads to liver fibrosis. However, the exact causes and mechanisms of the development of NASH remain unknown.

Recent investigations have suggested a “multi-hit process” model for the development of NASH [3]. Liver inflammation including NASH can be initiated or enhanced by multiple cytokines secreted mainly by Kupffer cells or macrophages [4]. During liver fibrogenesis, myofibroblasts, that are not present in normal liver, also contribute to liver fibrogenesis through the remodeling of extracellular matrix [5]. In pro-inflammatory cascades, there are several key factors that play a crucial role in initiating or halting inflammation. Tumor necrosis factor (TNF)-α is one of such key molecules, and anti-TNF-α therapies are used widely to treat human inflammatory disorders, such as rheumatoid arthritis and inflammatory bowel diseases [6,7]. To activate TNF-α, a membrane-bound pro-TNF-α must be appropriately and sufficiently cleaved by the prototypical sheddase, TNF-α-converting enzyme (TACE) [8]. Previously, we showed that nardilysin (N-arginine dibasic convertase; Nrd1), a zinc metalloendopeptidase of the M16 family that ubiquitously localizes in various organs, enhances the shedding of TNF-α through TACE activation [9–13]. Nardilysin binds to TACE and directly enhances its catalytic activity [10,11]. It also promotes the ectodomain shedding of


Received November 27, 2013; Accepted April 28, 2014; Published May 21, 2014

Copyright: © 2014 Ishizu-Higashi et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the Japan Science Promotion Society (JSPS) KAKENHI 21229009, 23300117, 23590937, 24229005, 24590914, 24590916, 24659363, 25112707, 25130706, 25461021, 25860533, and 26293173; Research program of the Project for Development of Innovative Research on Intractable Diseases, Hepatitis, and The innovative development and the practical application of new drugs for hepatitis B from the Ministry of Health, Labour and Welfare, Japan; the Funding Program for Next-Generation World-leading Researchers (LS05S), Grants-in Aid from the Ministry of Education, Culture, Science, Sports and Technology of Japan; Kobayashi Foundation for Cancer Research; The Naito Foundation; and Princess Takamatsu Cancer Research Foundation (13-24514). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: seno@kuhp.kyoto-u.ac.jp (HS); nishi@kuhp.kyoto-u.ac.jp (EN)
TNF-α, resulting in activation of the TNF-α/nuclear factor-κB pro-inflammatory signaling cascade [12].

In this study, we aimed to elucidate the mechanisms that distinguish NASH from simple liver steatosis. We examined the role of nardilysin, that is known to enhance TNF-α shedding, in the development of steatohepatitis using Nrd1<sup>+/+</sup> and Nrd1<sup>−/−</sup> mice fed a choline-deficient amino acid-defined (CDAA) diet and a high-fat diet (HFD), that are used widely to reproduce the natural course of NASH and liver fibrosis in mice as well as in humans.

Materials and Methods

Ethics statement

All animal experiments were undertaken in accordance with institutional guidelines. The Review Board of Kyoto University granted ethical approval for this study.

Animal models

Nardilysin-deficient (Nrd1<sup>−/−</sup>) mice (CDB0466K: http://www.cdb.riken.jp/arg/mutant%20mice%20list.html) were previously described [13]. Male Nrd1<sup>+/+</sup> and Nrd1<sup>−/−</sup> mice with the BL6/CBA background were bred and housed in a temperature- and light-controlled facility with unlimited access to food and water. To induce steatohepatitis and liver fibrotic changes, 10–12-week old male mice were fed a control choline-supplemented amino acid-defined (CSAA) diet or a choline-deficient amino acid-defined (CDAA) diet (Research Diets, New Brunswick, NJ, USA) for 4, 12, or 20 weeks according to the previous reports [14,15]. As another diet-induced model of steatohepatitis and liver fibrosis, mice were fed a HFD (Oriental Bio Service, Kyoto, Japan) for 20 weeks on the basis of previous studies [16,17]. Triglyceride levels in the livers were determined with Triglyceride Quantification kit (Abcam, Cambridge, MA, USA) according to the manufacturer’s protocol. Serum levels of alanine aminotransferase (ALT) were
measured using a Transaminase CII-Test Wako kit (Wako Pure Chemical Industries, Osaka, Japan).

**Histological analyses and immunostainings**

The liver was resected at various time points, fixed with 4% buffered paraformaldehyde solution, embedded in paraffin, and sectioned into 5-μm thickness. Oil red O (Wako Pure Chemical Industries) staining was performed to confirm fatty deposition. Sirius red (saturated picric acid containing 0.1% Direct Red 80 and 0.1% Fast Green FCF; Sigma-Aldrich, St. Louis, MO, USA) staining was done to visualize collagen deposition. Stained fibrotic areas were measured as percentage area in a representative high-power field in each mouse using Image J software. For the immunostainings the sections were incubated overnight with the primary antibodies at 4°C, after which the secondary antibodies were added. Kupffer cells or macrophages were stained with rat anti-F4/80 monoclonal antibody (Abcam). Negative controls were prepared with isotype IgG.

**Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)**

Total RNA was extracted using Trizol (Life Technologies, Carlsbad, CA, USA). Single-strand complementary DNA (cDNA) was synthesized using a Transcriptor First Strand cDNA Synthesis kit (Roche Applied Science, Basel, Switzerland). qRT-PCR was performed using SYBR Green I Master (Roche Applied Science) and Light Cycler 480 (Roche Applied Science). Values are expressed as arbitrary units relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primer sets used were: TNF-α-Forward, CCCTCAGCTCAGATCTCCTTCT, TNF-α-Reverse, GCTAGACGTGGGCTACAG; interleukin (IL) 6-Forward, TTGAACGCTTCGCGTCAG; interleukin (IL) 1β-Forward, TTTGAGGCTGGCGGTACCT, IL1-β-Reverse, ATGTTTGCGGCCTCGTCAA-CT; CCR2-Forward, ATGGCGGCTAGTATCGATC; CC-R2-Reverse, CGGCTACACATCACATG; collagen I-For-
ward, GCTCCCTTTAGGGCCACT, collagen I-Reverse, ATTCGGGAGCACTTAGGAC; collagen IV-Forward, TCCGGGATGAGATTGGTTTCC, collagen IV-Reverse, CTGGCCTATAAGCCCTGGT; tissue inhibitor of metalloproteinase (Timp) 1-Forward, CTTGGTTCCCTGGCGTACTC, Timp1-Reverse, ACCTGATCCGTCCACAAACAG; transforming growth factor β1-Forward, CTCCCGTGGCTTCTAGTG; TGF-β1-Reverse, GCCCTAGTTTGGAGGATCTG; α-SMA-Forward, GTCGCCAGACATCGGAGTAA; α-SMA-Reverse; TGGGATCCTCCGCTAGGA.

Measurement of cytokine levels by enzyme-linked immunosorbent assay (ELISA)

To determine the production and secretion of TNF-α protein in CDAA-treated mouse liver, a modified protocol that described in previous reports was used [18,19]. In brief, a liver fragment was divided into two specimens (100 µg each). One specimen was subjected directly to protein extraction, and the amount of protein extracted was determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). The other was cultured in a 24-well flat-bottomed culture plate in serum-free Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies) supplemented with penicillin and streptomycin (Life Technologies). After 12 hours, the supernatant was collected and the protein level measured. The amounts of TNF-α, IL6, and IL1-β proteins were measured using a Mouse ELISA Ready-SET-Go! kit (eBioscience, San Diego, CA, USA) according to the manufacturer’s protocol.

Mouse peritoneal macrophage experiments

Mouse peritoneal macrophages were isolated from 8-week-old female C57BL/6J mice. Peritoneal cells were harvested by peritoneal lavage with 10 ml PBS. Cells were re-suspended and

Figure 3. TNF-α was expressed in Nrd1+/+ and Nrd1−/− mice fed the CDAA diet. A. Immunohistochemistry showed that TNF-α protein (red, arrowheads) was expressed in F4/80-positive Kupffer cells or macrophages (green, arrowheads) in the livers of both Nrd1+/+ and Nrd1−/− mouse fed the CDAA diet for 20 weeks (right), but not in mice fed the CSAA diet for 20 weeks (left). A blue color indicates DAPI-positive nuclei. Bars indicate 50 µm. B. The number of F4/80-positive cells/×100 high-power field (HPF) in livers slightly increased (approximately 1.2 times) only in Nrd1+/+ mice fed the CDAA diet (right). C. Relative expression of mRNA are shown as relative values compared to those at 0 w. The mRNA expression level of CCR2 was increased in Nrd1+/+ mice fed the CDAA diet, and the levels were significantly higher than respective values in Nrd1−/− mice fed the CDAA diet. *P<0.05. doi:10.1371/journal.pone.0098017.g003
cultured in D-MEM supplemented with 10% FCS, 100 mg/ml of penicillin, 100 mg/ml of streptomycin, and 1.25 μg/ml of amphotericin B. 1.0×10⁶ peritoneal cells were seeded into a 48-well dish, and incubated for 2 hours. Then, cells were washed in PBS, and re-cultured in the serum-free medium. To inhibit TNF-α activity, either control serum or 0.4 mg/ml of anti-TNF-α neutralizing polyclonal antibodies (R&D systems) was administered into the culture medium. After 30 minutes later, 1 mg/ml of lipopolysaccharide (LPS) were added. Medium and cells were collected 2 hours after the stimulation, and subjected to the analyses according to the methods described above.

Statistical analyses

Results are the mean ± standard deviation unless stated otherwise. Differences between treatments, groups, and strains were analyzed using the two-tailed Student’s t-test.

Results

Nrd1+/− mice did not develop steatohepatitis with CDAA diet

The CDAA diet is deficient in choline only, but contains methionine, allowing observation of the sequential development of steatohepatitis and liver fibrotic changes in a longer experimental
Nrd1 was required for sufficient secretion of TNF-α

TNF-α is one of the key molecules that are involved in the development of NASH [4–7,20]. Because secretion of activated TNF-α is the initial step in nardilysin-mediated production of inflammatory cytokines [12], we hypothesized that sufficient secretion of TNF-α by nardilysin is required for the development of steatohepatitis. Thus, we aimed to ascertain whether TNF-α was produced and secreted sufficiently in the livers of Nrd1+/+ and Nrd1−/− mice fed the CDAA diet. qRT-PCR showed that the mRNA of TNF-α was increased in both Nrd1+/+ and Nrd1−/− mice fed the CDAA diet, and that in contrast to the results looking at IL6 and IL1-β mRNA levels, there was no significant difference between Nrd1+/+ and Nrd1−/− mice (Figure 2B). Immunohistochemistry showed that TNF-α protein was detected in F4/80-positive Kupffer cells or macrophages in both Nrd1+/+ and Nrd1−/− mice fed the CDAA diet for 20 weeks (Figure 3A, right, arrowheads). Conversely, TNF-α protein was barely detected in F4/80-positive Kupffer cells or macrophages in both Nrd1+/+ and Nrd1−/− mice fed the CSAA diet (Figure 3A, left). The number of F4/80-positive cells/100 high power field (HPF) in the liver was slightly increased only in Nrd1+/+ mice fed the CSAA diet but not in Nrd1−/− mice fed the CDAA diet and those
in *Nrd1<sup>+/+</sup>* and *Nrd1<sup>−/−</sup>* mice fed the CSAA diet (Figure 3B). qRT-PCR showed that mRNA expression of CCR2, a recruited macrophage marker, was significantly increased in *Nrd1<sup>+/+</sup>* mice, but not in *Nrd1<sup>−/−</sup>* mice (Figure 3C). This suggested that macrophages are not sufficiently recruited in *Nrd1<sup>−/−</sup>* mice. At 20 weeks of a CDAA feeding, production of TNF-α protein was significantly upregulated in both *Nrd1<sup>+/+</sup>* and *Nrd1<sup>−/−</sup>* mouse livers (Figure 4A, produced TNF-α), but the increase in TNF-α protein secretion from liver specimens into the conditioned medium was decreased significantly (0.46-fold) by *Nrd1* knockout (Figure 4A, secreted TNF-α). In contrast, production of IL6 and IL1-β proteins were not increased in *Nrd1<sup>−/−</sup>* mice fed a CDAA diet (Figure 4B). These data suggested that nardilysin was required for the shedding of TNF-α in mice fed the CDAA diet and possibly the induction of inflammation. To further investigate that possibility, we examined the effect of pre-incubation with anti-TNF-α neutralizing antibodies on the production of IL6 and IL1-β. Following LPS stimulation mRNAs and secreted proteins of both IL6 and IL1-β from macrophages were significantly increased, and administration of anti-TNF-α neutralizing antibodies significantly suppressed the production of IL6 and IL1-β (Figure 4C). This also suggested that TNF-α secretion played an important role to induce IL6 and IL1-β production in mice.

*Nrd1<sup>−/−</sup>* mice were resistant to CDAA diet-induced liver fibrotic changes

Persistent steatohepatitis results in hepatic fibrosis [1–4]. Using Sirius red staining we investigated whether secretion/production of inflammatory cytokines enhanced by nardilysin was associated with the development of liver fibrotic changes. Four weeks after CDAA feeding, fibrotic changes were not prominent in both *Nrd1<sup>+/+</sup>* and *Nrd1<sup>−/−</sup>* mice (Figure 5A and B). During CDAA diet administration, mRNA expression levels of collagen I, collagen IV, TIMP, TGF-β, and αSMA were significantly increased in the livers of *Nrd1<sup>+/+</sup>* mice but not in those of *Nrd1<sup>−/−</sup>* mice. Those factors were not altered by administration of the CSAA diet in *Nrd1<sup>+/+</sup>* or *Nrd1<sup>−/−</sup>* mice. *P*<0.05. doi:10.1371/journal.pone.0098017.g006
Discussion

In the present study, we demonstrated that steatosis was induced by the CDAA diet in both Nrd1+/+ and Nrd1−/− mice, although fatty changes were less prominent in Nrd1−/− mice. Importantly, steatohepatitis followed by liver fibrotic changes was observed only in Nrd1+/+ mice and not in Nrd1−/− mice. Secretion of TNF-α, and the production of inflammatory cytokines and fibrogenic factors were not upregulated in Nrd1−/− mice as compared with Nrd1+/+ mice. In the HFD model, steatohepatitis and liver fibrogenesis were hardly observed in Nrd1−/− mice. These data suggested that nardilysin plays an important role in the development of steatohepatitis followed by liver fibrosis.

In mice fed with the CDAA diet, the levels of hepatic triglyceride content were lower in Nrd1−/− mice compared with those in Nrd1+/+ mice, suggesting the possibility that nardilysin is involved in the regulation of hepatic lipid synthesis. A decreased steatosis in Nrd1−/− mice may partly affect hepatic inflammation. However, steatosis did occur in the liver of Nrd1+/+ mice; on the other hand, hepatic inflammation was not observed despite the presence of steatosis in Nrd1+/+ mice. This indicated that nardilysin has an important role in the initiation and/or promotion of inflammatory responses induced by the CDAA diet. Persistent inflammation distinguishes steatohepatitis from simple hepatic steatosis [1–3]. Among pro-inflammatory factors, TNF-α is one of the key molecules that initiate inflammatory cascades, and its role in the progression of NASH has been discussed [4–7]. For example, apoptotic change in the liver, which contributes to the progression of NASH, is inhibited by an anti-TNF receptor neutralizing antibody or pentoxifylline in a mouse model of NASH [20]. The absence of TNFR1, a receptor for TNF-α, reduces IL6 mRNA production in the liver fed with the HFD even in the presence of elevated serum TNF-α [21]. The absence of TNFR1 also reduces liver lipid accumulation and macrophage accumulation in livers of HFD-fed mice [21]. Thus, inhibition of TNF-α signaling appears to plays a pivotal role to suppress inflammatory reactions in NASH as well as other inflammatory disorders [22]. Although clinical application of anti-TNF-α therapy has not been established in the treatment of human NASH, anti-TNF-α neutralizing antibodies are effectively used to treat various human...
inflammatory disorders, such as rheumatoid arthritis and inflammatory bowel diseases [6,7]. We previously reported that nardilysin is essential for the sufficient activation of TNF-α in cooperation with TACE [10–12]. By the knockdown of Nrd1, TNF-α secretion is decreased concomitantly with decreased TACE activity, and the production of inflammatory cytokines such as IL6 and IL1β is significantly suppressed [10–12]. In the present study, it is worth noting that TNF-α secretion from liver specimens was decreased significantly in Nrd1−/− mice fed the CDAA diet, while TNF-α production was not different between Nrd1+/+ and Nrd1−/− mice fed the CDAA diet. Consistently, the production of various inflammatory cytokines were not increased in the livers of Nrd1−/− mice. Although the precise mechanism of the decreased inflammatory responses in Nrd1−/− mouse livers was not clear, it appeared likely that the impaired release of TNF-α in Nrd1−/− mouse livers was one of the reasons for the reduced inflammatory reactions in Nrd1−/− mice. It would be also possible that different activation status of TNF-α and inflammatory responses conversely affect difference of fatty contents between Nrd1+/+ and Nrd1−/− mice. Whatever the case, nardilysin seemed to play an important role in the development of steatohepatitis and liver fibrosis presumably through TNF-α activation.

Previous studies have shown that Kupffer cells and recruited macrophages interact with hepatic stellate cells, accelerate their activation, and promote the fibrogenic responses [4,17]. Activated myofibroblasts also promote the remodeling of the extracellular matrix and contribute to liver fibrosis [5]. Indeed, our immunohistochemical analyses showed that Kupffer cells and macrophages were major producers of TNF-α in the livers of mice fed the CDAA diet, and that αSMA-positive myofibroblasts were not prominent in Nrd1−/− mice. Decreased release of TNF-α from Kupffer cells and recruited macrophages could be one of the mechanisms for the suppression of diet-induced steatohepatitis in Nrd1−/− mice, and thus nardilysin in Kupffer cells and recruited macrophages may be required for the progression of liver fibrosis, concomitantly with the recruitment of myofibroblasts. However, we could not completely exclude the possible

Figure 8. Liver fibrogenesis was not observed in Nrd1−/− mice fed the HFD. A. Steatosis was observed in both Nrd1+/+ and Nrd1−/− mice after 20-week HFD administration (right), but not in those fed a normal control diet (left). Bars indicate 100 μm. B. Quantification of triglyceride in the liver. Triglyceride was elevated in the livers of both Nrd1+/+ and Nrd1−/− mice after 20-week HFD administration, although it was significantly higher in Nrd1+/+ mice. n = 4, each. *P<0.05. C. Serum ALT levels were significantly elevated in Nrd1+/+ mice upon administration of the HFD, but were not elevated in other mouse groups. *P<0.05. D. Fibrotic area was less prominent in Nrd1−/− mice than in Nrd1+/+ mice (right). Bars indicate 100 μm. E. Fibrotic area was observed only in the livers of Nrd1+/+ mice fed the HFD (right). n = 5, each. *P<0.05. doi:10.1371/journal.pone.0098017.g008
contribution of nardilysin in other cells such as hepatocytes or endothelial cells for the development of NASH and liver fibrosis. Therefore, genetically-engineered mice lacking or strongly expressing nardilysin in Kupffer cells and macrophages may be required to confirm our hypothesis in future studies.

In summary, the present study indicates that nardilysin contributes to the development of diet-induced NASH and liver fibrotic changes by regulating chronic liver inflammation. Nardilysin could be an attractive target for anti-inflammatory therapy against NASH and liver fibrosis.

Author Contributions

Conceived and designed the experiments: SIH HS. Performed the experiments: SIH YM KI MT Yoshito Kimura YT Yuto Kimura YN KK. Analyzed the data: SIH HS KK. Contributed reagents/materials/analysis tools: EN HK. Wrote the paper: SIH HS TC.

References


Figure 9. Inflammatory and fibrogenic factors were not increased in Nrd1<sup>−/−</sup> mice fed the HFD. A. mRNA of TNF-α was slightly increased in both Nrd1<sup>−/−</sup> (significantly) and Nrd1<sup>+/+</sup> mice. In contrast to Nrd1<sup>−/−</sup> mice, the mRNA expression level of IL1-β was not increased in Nrd1<sup>−/−</sup> mice. *P<0.05. B. mRNA expression levels of collagen I, collagen IV, TIMP, TGF-β, and αSMA in the livers of Nrd1<sup>+/+</sup> mice fed a HFD for 20 weeks were significantly higher than the respective values of Nrd1<sup>−/−</sup> mice fed the control diet. However, they were not altered by HFD in Nrd1<sup>−/−</sup> mice. *P<0.05. doi:10.1371/journal.pone.0098017.g009

Nardilysin could be an attractive target for anti-inflammatory therapy against NASH and liver fibrosis.


