•ORIGINAL RESEARCH ARTICLE•

The Level of Nesfatin-1 in a Mouse Gastric Cancer Model and Its Role in Gastric Cancer Comorbid with Depression

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Background: The incidence of depressive symptoms is higher in cancer patients. And depression can also affect the occurrence, development and outcome of cancer through the neuroendocrine-immune-network system.

Objective: To study the level of Nesfatin-1 in the plasma and brain tissue and its role in the pathogenesis in gastric cancer comorbid with depression using a mouse gastric cancer model.

Methods: 18 mice were randomly divided into the normal control group (NCG), gastric cancer without stress model group (GCNS), and gastric cancer combined with stress model group (GCS). The mice of the GCNS group were inoculated subcutaneously with mouse forestomach carcinoma (MFC) after 5 weeks of nomal feeding to establish a model of subcutaneous transplantation tumor. After 5 weeks of chronic unpredicted mild stress (CUMS) in the GCS group, subcutaneous inoculation of MFC was used to establish a subcutaneous transplantation tumor model for 1 week. Evaluation of mice behavior was performed by open field test, sucrose preference test and forced swimming test (FST). Determination of Nesfatin-1 concentration in plasma and brain tissue was carried out using enzyme linked immunosorbent assay (ELISA) and Western Blot.

Results: The weight increment in the GCS group was significantly lower than that in the GCNS group (t=-3.39, p<0.001). And both GCS and GCNS were lower than the NCG group (t=-6.33, p<0.001; t=-2.94, p=0.01). In the open field test, the horizontal moving distance of the GCS group was less than that of the GCNS group (t=-2.50, p=0.025), and both GCS and GCNS were smaller than the NCG group (t=-5.87, p<0.001; t=-3.38, p=0.004). The dead time of the GCS group was longer than that of the GCNS and the NCG groups (t=2.56, p=0.022; t=3.84, p=0.002). The Nesfatin-1 level in the middle brain, hippocampus and plasma was significantly higher in NCG group and GCS group than in the GCNS group. The concentration of Nesfatin-1 in the GCS group was significantly higher than that in the NCG group.

Conclusions: There is a decrease of Nesfatin-1 level in brain tissue and plasma in mice with gastric cancer without stress. CUMS stress can induce depressive behavior in gastric cancer mice, and increase the level of Nesfatin-1 in brain tissue and plasma. Therefore, Nesfatin-1 may be related to the pathogenesis of gastric cancer stress related depression.

Key words: Nesfatin-1; gastric cancer; depression; stress

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1. Introduction

Gastric cancer is one of the most common malignant tumors seen in clinical practice. The incidence of gastric cancer combined with depression is 27% to 44%, which is far higher than other cancers. Among them, the incidence of depression in female cancer patients is higher than that of men.[1,2] Studies have shown that the negative mood of depression can increase the incidence of cancer through a disorder of neuroendocrine function and a decrease in the immune function of the body, and promote the progress of cancer. However, its specific mechanism is not yet clear.[3] In recent years, Nesfatin-1, as a newly discovered appetite suppressor, has received extensive attention in the research of gastric cancer and depression. Studies have found that they are widely expressed in the central and peripheral areas, and play an important role not only in inhibiting food intake, but also in the pathogenesis of cancer and depression.[4-6]

Nesfatin-1, a new appetite suppressor found by the Japanese scholar OH-I in 2006, plays an important role in feeding, sugar metabolism, and energy balance. In rats, a cerebral ventricular injection of Nesfatin-1 caused a dose dependent reduction in NUCB2 mRNA within 6 hours, whereas a reinjection of the Nesfatin-1 antibody Ab24 returned rat feeding back to normal.[7,8] Nesfatin-1 has been found to play an important role in emotional regulation and cancer. The clinical study of depression showed that the level of plasma Nesfatin-1 in patients with depression was significantly higher than that in the normal control group, and was positively correlated with the HAM-D score.[9] Our previous studies also showed that the plasma Nesfatin-1 of the depressive model rats was significantly higher than that of normal rats.[4] Other animal studies showed that intracerebroventricular injection of Nesfatin-1 could significantly induce depression in rats, increase anxiety and fear behaviors in dose-dependent manner, and reduce exploratory behavior. [10,11] In addition, studies have found that Serum Nesfatin-1 levels in patients with advanced lung cancer with weight loss were lower than those in normal and non-weighted lung cancer patients.[12] In addition, studies have shown that Nesfatin-1 can inhibit the proliferation of human ovarian cancer cell lines by inducing cell apoptosis through mTOR and Rho / ROCK signaling pathways.[13] And our previous clinical studies found that the plasma Nesfatin-1 concentration in patients with gastric cancer and depression is significantly lower, and is associated with the incidence of gastric cancer with depression and the severity of depression.[5] However, it has not been verified in animal experiments, and its specific mechanism is not yet clear. This study intends to explore the level of Nesfatin-1 in plasma and brain tissue of mouse gastric cancer models and its mechanism of action in the comorbidity of depression in gastric cancer through animal experiments.

2. Methods

2.1 Laboratory animals

18 mice in the inbred line, female, SPF, mean (sd) weight 18(2) g, 6-7 weeks old, purchased from the Institute of Hematology, Chinese Academy of Medical Sciences. Laboratory animal production license: SCXK (津) 2015-0001. During the experiment, the experimental animal room (at Renmin Hospital of Wuhan University) conditions were the following: free supply of standard feed and clean tap water were fed,12 h light/12 h dark circadian rhythm, room temperature 23-25C, humidity (55 + 10)%. All operations were approved by the Ethics Committee of Wuhan University Renmin Hospital.

2.2 Reagents and materials

Mouse forestomach carcinoma (MFC) were purchased from the cell bank of the Chinese Academy of Sciences. RPMI-1640 culture was purchased from the Gibco of the United States. Fetal bovine serum was purchased from Zhejiang Tianhang Biotechnology Co. Ltd. Trypsin, penicillin and streptomycin were purchased from Shanghai Genom Biotechnology Co. Ltd. The mouse Nesfatin-1 Elisa Kit (CEA242Mu) was purchased from Wuhan Youo Technology Development Co. Ltd. Rabbit anti-Nesfatin-1 polyclonal antibody was purchased from the Sigma Company. Horseradish peroxidase (HPR) tagged goat anti-rabbit secondary antibody was purchased from the Biosharp Company.

2.3 Instruments

We used an animal track record analyzer (ethovision3.0 Netherlands), CO_2 Incubators (CB150 Binder, Germany), benchtop (AIRTECH), inverted microscope(IX51 Olympus, Japan), and ChemiDoc Touch imaging system (Bio-Rad).

2.4 Animal grouping

18 mice were randomly divided into 3 groups: normal control group (NCG), gastric cancer without stress model group (GCNS), and gastric cancer combined with stress model group (GCS), 6 rats in each group. Mice in the NCG group were reared ordinarily. GCNS mice were vaccinated subcutaneously after 5 weeks of nomal feeding with mouse forestomach carcinoma (MFC) to establish a subcutaneous xenograft model for 1 week. GCS mice were given 5 weeks of chronic unpredictable mild stress (CUMS), then we performed subcutaneous vaccinations with MFC to establish a subcutaneous xenograft model for 1 week.

2.5 Laboratory method

2.5.1 Chronic unpredicted mild stress (CUMS)

Based on Willner's Stress improvement, the GCS group received 24h fasting, 24h water withdrawal, 24h isolation, 5min ice water (4°C), 5min hot water (43°C), 10min restraint, 1min at the end of the clip (the position of the clip is placed at the end of the tail tip of the mouse 1cm) and moisture for 24 hours.[4] 1-2 stimuli were randomly selected each day. The same stimulus was discontinuous, and each cycle was 7 days.[14] When the difference between the GCS and the other two

groups was statistically significant, it showed that the depression model mice were successfully established. Then a tumor was inoculated and observed for 1 week.

2.5.2 In vitro culture and inoculation of tumor cells

Mice gastric cancer cell MFC was inoculated in a culture bottle at the appropriate concentration , added into RMPI-1640 culture medium (containing 10% fetal bovine serum, 1% green, streptomycin), and then cultured in 5% CO_2 incubator. The liquid was changed once every other day and passed every 3-4 days. After passage, 0.2 ml of trypsin and 1.8 ml of PBS were added and digested for 30 seconds. The cell suspension was made by blowing into the medium containing fetal bovine serum. After centrifugation, it was diluted with PBS to a concentration of (1x107/ml). The MFC cell suspension 0.2ml (containing 2X106 of gastric cancer cells) was planted in the subcutaneous of the back of the GCNS group and the GCS group.

2.5.3 Behavioral test

(1) Sugar water preference test: The sugar water consumption test was given before and after the model. All mice were given 1% sucrose solution (W/V) for 24 hours before the experiment (2 bottles per cage), and then a bottle of 1% sucrose solution was replaced by normal drinking water for 12 hours, then two bottles of water were exchanged for 12 hours, and the last 24 hours were fasted. At the beginning of the experiment, 1% bottles of sucrose solution were placed in a bottle, the other was placed in the normal drinking water. After 1 hours, two bottles were interchanged (eliminating the unilateral preference of mice). The calculation of 2h sucrose preference value (%) = consumption of sucrose solution / total liquid consumption * 100%. (2)Open field test: open field 50cm long, 50cm wide, 35cm high, the bottom is painted white, with white and transparent walls. Mice slowly enter into the open field and recording and analysis of their trajectory for 5 mins with Ethovision 3.0 was performed. (3) Forced swimming test: The mice were placed in a cylindrical bucket (high 50cm, diameter 20cm, water depth 25cm and the temperature was [25 + 2 ℃].[15]) and forced to swim for 6 mins, with recording of the last 4 minutes.

2.5.4 Specimen collection and ELISA

After completion of behavioral tests, mice were anesthetized with 1% pentobarbital (35 mg/kg) intraperitoneally. Blood was taken from the heart and placed in an EDTA anticoagulant tube, then centrifuged to obtain a plasma sample and stored in a deep freezer at -80°C. Determination of Plasma Nesfatin-1 Concentration was done in accordance with ELISA Reagent instructions.

2.5.5 Western Blot

Immediately after blood was drawn from the heart, the brain was decapitated and the hippocampus and midbrain were quickly peeled off on ice. The appropriate amount of brain tissue was added to RIPA protein lysate, and homogenized on ice to extract total tissue protein. After BCA detection of protein concentration, we added sample buffer and boiling water bath for 10 min to make protein denaturation. We took 20 μg of total protein for SDS-PAGE gel (5% gel, 10% gel). The transmembrane was carried out after the end of electrophoresis. After the membrane was transferred, the PVDF membrane was transferred to a TBST blocking solution containing 5% non-fat dry milk powder and closed for 1 h in a shaker at room temperature. The membrane was soaked in the primary antibody after TBST washing (Rabbit anti-Nesfatin-1 1:1000 dilution). After incubation in a shaker overnight, TBST was washed 3 times for 10 min each time, and then incubated in HPR-labeled secondary antibody (1:10000 dilution). Then it was incubated for 1h at room temperature, and the membrane was washed and exposed using the ECL chemiluminescence method. The protein bands were analyzed by Image ab for gray value analysis. Nesfatin-1/GAPDH gray value ratio is the relative expression of Nesfatin-1.

2.5.6 Denudation of tumor tissue

After removing the brain, the tumor tissue was removed and then weighed.

2.6 Statistical treatment

Data was processed with SPSS 19.0. Measurement of normality of measurement data was done by Kolmogorov Smirnov Test. The form of data that is normally distributed is represented by X(S). Independent sample T test was used among the two groups, and single factor analysis of variance was used for the comparison among the three groups and above (The LSD-t test was used for the comparison between groups) $p<0.05$ was considered statistically significant.

3. Results

3.1 Behavioral test results

Before modeling, there was no significant difference between the three groups in body weight, or sugar water preference test ($F=0.11$, $p=0.896$; $F=0.78$, $p=0.476$) (Table 1). After the modeling of GCNS and GCS, the weight increment, horizontal distance and erect times of the GCS group were significantly lower than that of the GCNS group ($t=-3.39$, $p<0.001$; $t=-2.50$, $p=0.025$; $t=-2.61$, $p=0.02$). And the two groups of GCS and GCNS were all lower than the NCG group (GCS&NCG; $t=-6.33$, $p<0.001$; $t=-5.87$, $p<0.001$; $t=-5.59$, $p<0.001$; GCNS&NCG; $t=-2.94$, $p=0.01$; $t=-3.38$, $p=0.004$; $t=-2.98$, $p=0.009$); The dead time of the GCS group was longer than that of the GCNS group and the NCG groups ($t=2.56$, $p=0.022$; $t=3.84$, $p=0.002$). In the GCNS group there was only a higher trend compared with the NCG group, but the difference was not statistically significant ($t=1.27$, $P=0.222$) (Table 2).

Figure 1. The flowchart of the study

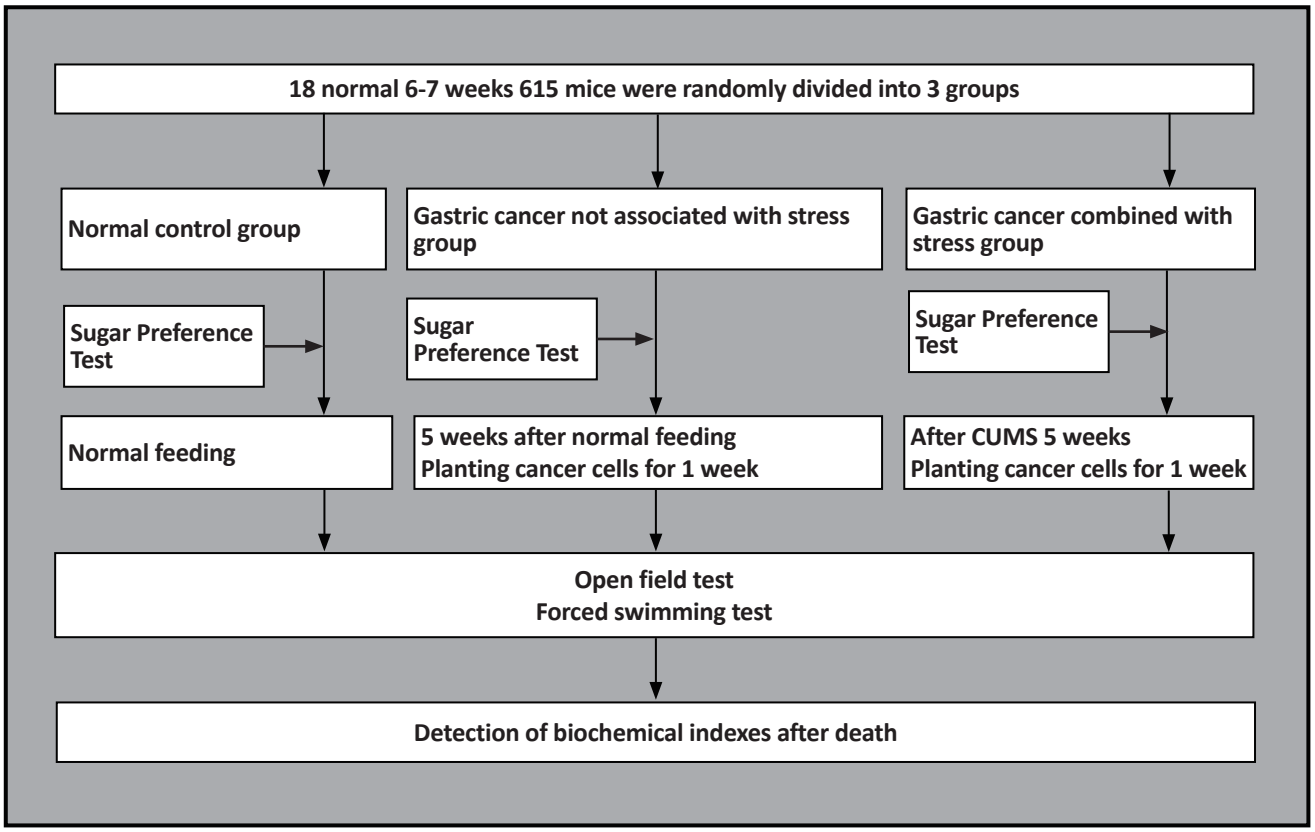

Table 1. Pre-modelling mouse behavior evaluation (mean(SD))

Group	Quantity	Weight(g)	Sugar Preference Index
NCG		17.82(1.30)	0.61(0.12)
GCNS	6	18.03(0.83)	0.50(0.19)
GCS	6	17.75(1.11)	0.60(0.16)
F	6	0.11	0.78
P		0.896	0.476

Comparison of three groups and above by single factor analysis of variance (Pairwise comparison using LSD-t test)

Table 2. Post-model mouse behavior evaluation (mean(SD))

Group	Quantity	Increased Weight (g)	Dead Time(s)	Open Field Test	
				Horizontal Distance	Upright Time
NCG		6.22(1.14) $^{\triangle}$	64.47(30.21)	3689.51(1063.38) $^{\triangle\triangle}$	48.83(9.50) $^{\triangle\triangle}$
GCNS	6	4.03(1.76)*	85.17(30.45)	2270.77(441.69)**	31.67(9.56)**
GCS	6	1.52(0.75)$^{**\triangle\triangle}$	126.82(23.15)$^{**\triangle\triangle}$	1222.28(511.78)$^{**\triangle}$	16.67(10.80)$^{**\triangle}$
F	6	20.07	7.64	17.38	15.63
P		0.000	0.005	0.000	0.000

*: Compared with NCG, *p<0.05，**p<0. 01; $^{\triangle}$: Compared with GCNS, $\triangle$ p<0.05, $\triangle\triangle$ p < 0.01
Comparison of three groups and above by single factor analysis of variance (Pairwise comparison using LSD-t test)

3.2 Comparison of Nesfatin-1 expression and tumor weight in plasma, hippocampus and midbrain between groups

The concentration of plasma, hippocampus and midbrain Nesfatin-1 in the NCG group and GCS group was significantly higher than that in the GCNS group. (NCG&GCNS: t=3.46, p=0.003; t=4.58, p<0.001; t=4.18, p=0.001; GCS&GCNS: t=6.04, p<0.001; t=8.84, p<0.001; t=8.16, p<0.001), and the plasma, hippocampal and midbrain Nesfatin-1 concentrations in the GCS group were significantly higher than those in the NCG group (t=2.58, p=0.021; t=4.26, p=0.001; t=3.97, p=0.001)(Figure 2, Table 3). There was no significant difference in tumor weight between the GCS group and the GCNS group (t=-0.301, p=0.770).

4. Discussion

4.1 Main findings

This study found that mice in the GCS group exhibited depressive states (reduced exploration activity, bradykinesia, behavioral despair) consistent with psychomotor changes, loss of interest or pleasure in human depression. Nesfatin-1 in the middle brain, hippocampus or plasma, group GCS was significantly higher than that of group NCG and GCNS, and the GCNS group was significantly lower than the NCG group. This suggests that Nesfatin-1 has a certain role in the pathogenesis of cancer and cancer comorbid with stress depression.

The results from the open field experiment of the GCNS group mice were also significantly different from that of the NCG group. It may be due to neuroendocrine disorders caused by gastric cancer leading to cancer fatigue. Nesfatin-1 in the middle brain, hippocampus or plasma, in the GCS group was significantly higher than that of the NCG and GCNS group, and the GCNS group had a significantly lower amount than the NCG group. This indicates that Nesfatin-1 has a certain significance in the pathogenesis of cancer and cancer comorbid with depression.

Compared with our previous study, the increase of Nesfatin-1 levels in the plasma of depressive rats induced by chronic stress of CUMS was consistent, indicating that the state of depression caused by chronic stress is related to the level of plasma Nesfatin-1.[4] The open field test in the GCNS group of mice was also significantly different from that in the NCG group. This may be due to cancer-related fatigue from neuroendocrine disorders caused by gastric cancer, and does not exclude the possibility of cancer comorbid with depression. However, our group's preliminary clinical study found that the plasma concentration of Nesfatin-1 in patients with gastric cancer and depression is significantly reduced, presumably because the clinical study of gastric cancer patients are mostly advanced gastric cancer. Its pathological state may break the relationship between depression and Nesfatin-1.[5] Is this a coincidence that the results are consistent with the results of the GCNS group in this study? Or is gastric cancer itself a cause of comorbid with depression, and not just gastric cancer, a stressful factor that can cause comorbid disease and depression? It is also possible that the mechanism of Nesfatin-1in the gastric mice which also suffered from stress in this study. This is different from the mechanism of action of emotional

Figure 2. Three groups of mice expressed bands in the hippocampus and midbrain

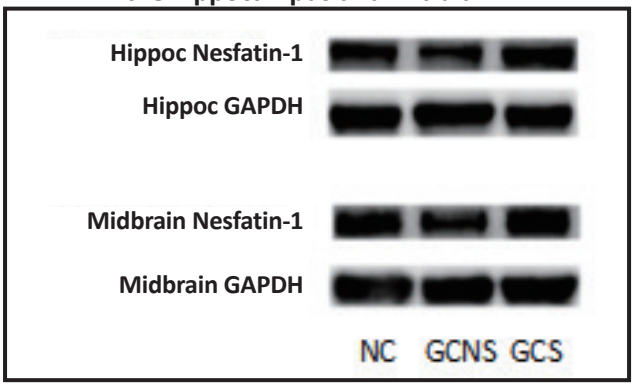

Table 3. Levels of plasma and brain Nesfatin-1 and tumor weight in each group (mean(SD))

Group	Number	Plasma Nesfatin-1 (ng/ml)	Hippocampus Nesfatin-1 Relative Transcript Level	Midbrain Nesfatin-1 Relative Transcript Level	Tumor Weight (g)
NCG		7.65(0.79) $^{\triangle\triangle}$	0.99(0.09) $^{\triangle\triangle}$	0.99(0.05) $^{\triangle\triangle}$	-
GCNS	6	4.68(2.02)**	0.45(0.20)**	0.65(0.11)**	0.99(0.21)
GCS	6	9.85(1.37)$^{*\triangle\triangle}$	1.49(0.28)$^{**\triangle\triangle}$	1.30(0.21)$^{**\triangle\triangle}$	1.03(0.26)
F/t	6	18.41	39.06	33.29	-0.301
P		<0.001	<0.001	<0.001	0.770

*: Compared with NCG, *p < 0.05, **p < 0.01; $^{\triangle}$: Compared with GCNS, $\triangle p$ < 0.05, $\triangle\triangle p$ < 0.01
Comparison of three groups and above by single factor analysis of variance (Pairwise comparison using LSD-t test)

disorders caused by gastric cancer, which is very worthy of in-depth research. The plasma Nesfatin-1 in the GCNS group was significantly lower than that in the NCG group, which is also consistent with the previous study. This may be due to the fact that cancer often leads to a decrease in food intake, weight loss, and decrease of body adipose tissue. The expression of Nesfatin-1 in adipose tissue is one of the sources of circulating Nesfatin-1.[5,16] It is possible that it leads to Nesfatin-1 reduction in gastric cancer. The weight gain of GCNS group was lower than NCG in this study, prompting this possibility. However, the weight gain of the GCNS group is higher than that of the GCS group. The stress prevents the decrease of Nesfatin-1 in gastric cancer, whereas it increases it. So it is not accurate to explain the effect on Nesfatin-1 by the amount of fat alone. It has yet to be further studied. Studies have shown that NUCB2/Nesfatin-1 can promote colon cancer cell migration, invasion, and epithelial-mesenchymal transition through LKB1, AMPK/TORC1/ZEB pathways.[17] A study by Yoo et al. showed that Nesfatin-1 can induce prostate cancer cell metastasis by autocrine.[18] In this study, the down-regulation of Nesfatin-1 in the central and peripheral regions of the GCNS group may also have protective implications for the prevention of metastasis. However, studies have shown that Nesfatin-1 can inhibit the proliferation of human ovarian cancer cell line HO-8910 by inducing apoptosis through mTOR and Rho/ROCK signaling pathways.[13] In this way, the downregulation of Nesfatin-1 caused by gastric cancer is not conducive to inhibiting the proliferation of cancer cells. Indicating that it may play a role in the development of gastric cancer. However, in our study, a significant elevation of the central and peripheral Nesfatin-1 in the GCS group may have lead to invasion and migration of cancer cells. The time of our model was relatively short, but there was no cancer metastasis, and there was no significant difference in the weight of the two groups. Whether stress-induced increase in Nesfatin-1 is also beneficial for inhibiting the proliferation of cancer is worth further study.

Studies have shown that Nesfatin-1 coexpresses with 5-hydroxytryptamine (5-HT) and norepinephrine (NE) in the midbrain nucleus raphe nucleus.[6] Yoshida et al. suggested that Nesfatin-1 activates 5-HT neurons in the stress-sensitive raphe nuclei and NE neurons in the locus coeruleus, thereby stimulating the corticotropin-releasing hormone (CRF) neurons in the paraventricular nucleus, activating the HPA axis. Studies have shown that HPA axis disorder may be associated with the occurrence of depression and anxiety in cancer patients.[19,20] Early clinical study showed that plasma cortisol in the patients with comorbid gastric cancer was significantly higher than that of the patients with gastric cancer without depression. The group of gastric cancer without depression was significantly lower than that of the healthy group, and the change of Nesfatin-1 in the brain and plasma in this study was in accordance with this study. This further suggests that Nesfatin-1 may be involved in the pathogenesis of gastric cancer and depression through 5-HT, NE, and the HPA axis.[5] Studies have shown that continuous intraperitoneal injection of Nesfatin-1 within 3 weeks resulted in decreased expression of BDNF protein in rat hippocampus and PFC.[11] The results of this study also showed that the expression of Nesfatin-1 in the hippocampus of stress depression mice was increased. These results provide evidence that Nesfatin-1 can reduce the exploratory behavior of animal models and induce anxiety like behavior. Its mechanism may be related to the BDNF protein in the hippocampus.

4.2 Limitations

This study has the following limitations: (a)The sample size is small and all of them are female. It can not be concluded whether Nesfatin-1 has a sex difference in the role of gastric cancer comorbid depression. The results need to be further verified. (b)Because of the limited experimental conditions, we used different behavioral measures before and after modeling. So we could not compare changes in behavior before and after modeling. (C)This study only set up a normal control group, gastric cancer without stress group, gastric cancer with stress group, but no simple depression group was established. So it is difficult to prove the change of Nesfatin-1 in the gastric cancer with stress group and simple depression group. This requires further study.

4.3 Implications

In summary, this study showed that the mesencephalon, hippocampal and plasma Nesfatin-1 were changed in the mice model of gastric cancer with stress depression or without stress depression. This shows that it has a certain role in the disease stress of gastric cancer and the incidence of depression and gastric cancer. This provides a new clue to the study of cancer and its comorbid stress depression: Prevalence of cancer comorbid depression is clinically high.[21] While gastric cancer is a life-threatening disease, cancer itself is a major stress factor. Whether the cancer comorbid with depression is the result of the pathology of cancer or the result of stress factors is difficult to say. Whether different changes of GCs and GCS in Nesfatin-1 can be used as markers for differential diagnosis of cancer comorbid stress depression or non-stress depression remains to be seen. But this study provides a reference for future research. The causal relationship between Nesfatin-1 and comorbid depression in cancer and its mechanism of action are not yet clear and require further study.

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Conflict of interest statement

All authors declare no conflicts of interest related to this study.

Animal ethics

All operations were approved by the Ethics Committee of Wuhan University Renmin Hospital.

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Authors' contribution

Nan Zhang was responsible for the design and implementation of this study, including statistics and writing of post-data; Huiling Wang and Xiao Ling provided revisions and input on the paper; Yanyan Wei and Jing He participated in the breeding and drawing of animals in this study; Gaohua Wang and Jiangbo Li provided guidance for research design and paper writing.

小鼠胃癌模型 Nesfatin-1 水平及其在胃癌共病抑郁发病中的作用

张楠，李江波，王惠玲，肖玲，魏艳艳，何静，王高华

背景：癌症患者伴有抑郁症状的发生率较高，而抑郁情绪又会通过神经 - 内分泌 - 免疫网络系统来影响癌症的发生、发展及转归。

目的：探讨小鼠胃癌模型血浆及脑组织中 Nesfatin-1 水平及其在胃癌共病抑郁发病中的作用机制。

方法：将 18 只 615 小鼠随机分为正常对照组（NCG）、胃癌不合并应激抑郁模型组（GCNS）、胃癌合并应激抑郁模型组（GCS）。GCNS 组小鼠饲养 5 周后于皮下接种小鼠前胃癌细胞（MFC）建立皮下移植瘤模型，GCS 组给以 5 周慢性不可预见应激（CUMS）后，皮下接种 MFC 建立皮下移植瘤模型 1 周。用旷场实验、蔗糖偏好试验、强迫游泳试验（FST）评价小鼠的行为。分别用酶联免疫吸附法（ELISA）及 Western Blot 技术来检测血浆及脑组织中 Nesfatin-1 浓度。

结果：GCS 组体重增量加显著低于 GCNS 组 (t = -3.39, p < 0.001)、且 GCS、GCNS 两组均低于 NCG 组 (t = -6.33, p < 0.001; t = -2.94, p=0.01)；旷场试验中 GCS 组水平移动距离小于 GCNS 组（t = -2.50, p = 0.025），且 GCS、GCNS 两组二者均小于 NCG 组（t = -5.87, p < 0.001; t = -3.38, p = 0.004）；GCS 组不动时间长于 GCNS 组与 NCG 组 (t = 2.56, p = 0.022; t = 3.84, p = 0.002)。中脑、海马及血浆 Nesfatin-1 水平 NCG 组与 GCS 组均显著高于 GCNS 组，且而 GCS 组 Nesfatin-1 浓度显著高于正常对照组，结果均有统计学意义。

结论：胃癌不合并应激抑郁小鼠脑组织及血浆 Nesfatin-1 水平降低，CUMS 应激可以引起胃癌小鼠的抑郁样行为，且可以升高脑组织及血浆 Nesfatin-1 水平，因此 Nesfatin-1 可能与胃癌及胃癌相关应激抑郁的发病有关。

关键词：Nesfatin-1; 胃癌；抑郁；应激

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