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RESEARCH ARTICLE

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Norepinephrine may promote the progression of *Fusobacterium nucleatum* related colorectal cancer via quorum sensing signalling

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ABSTRACT

Fusobacterium nucleatum (F. nucleatum) is closely correlated with tumorigenesis in colorectal cancer (CRC). We aimed to investigate the effects of host norepinephrine on the carcinogenicity of *F. nucleatum* in CRC and reveal the underlying mechanism. The results revealed that both norepinephrine and bacterial quorum sensing (QS) molecule auto-inducer-2 (AI-2) were positively associated with the progression of *F. nucleatum* related CRC ($p < 0.01$). *In vitro* studies, norepinephrine induced upregulation of QS-associated genes and promoted the virulence and proliferation of *F. nucleatum*. Moreover, chronic stress significantly increased the colon tumour burden of $Apc^{Min/+}$ mice infected with *F. nucleatum* ($p < 0.01$), which was decreased by a catecholamine inhibitor ($p < 0.001$). Our findings suggest that stress-induced norepinephrine may promote the progression of *F. nucleatum* related CRC via bacterial QS signalling. These preliminary data provide a novel strategy for the management of pathogenic bacteria by targeting host hormones-bacterial QS inter-kingdom signalling.

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Stress; norepinephrine; *Fusobacterium nucleatum*; colorectal cancer; autoinducer-2

Introduction

It has been estimated that over 100 trillion microbes reside in the human intestine as symbionts, and that they are mutually beneficial to the host [1]. However, dysregulation of the gut microbiota is related to a variety of diseases, including colorectal cancer (CRC), which is the third leading cause of cancer-related deaths worldwide [2]. An increasing body of evidence has demonstrated that *F. nucleatum*, previously recognized as a major constituent of the normal oral microbial community, is enriched in tumour tissues and stools of CRC patients compared to healthy controls [3–6]. An ectopic accumulation of *F. nucleatum* leads to a proinflammatory microenvironment that promotes CRC cellular proliferation [6,7]. *F. nucleatum* adhere to and invade epithelial cells via *fadA* and promotes CRC by modulating E-cadherin/β-catenin signalling [8–10]. Besides, *F. nucleatum* is capable of stimulating LC3-II expression, autophagic flux, and autophagosome synthesis in CRC cells which results in chemoresistance [11]. As an opportunistic pathogen, *F. nucleatum* also exists in healthy gut [12]. However, the mechanism that determines the pathogenicity of *F. nucleatum* are not entirely clear.

Interbacterial communication relies on the quorum sensing (QS) system, which utilizes signal molecules termed autoinducers (AIs) to regulate bacterial behaviour such as growth, virulence, and pathogenicity [13,14]. As a major molecule of QS, autoinducer-2 (AI-2), a furanosyl borate diester or tetrahydroxy furan [15], has been reported to be recognized by many Gram-negative and Gram-positive bacteria [16] and is closely correlated with the pathogenicity of a variety of bacteria in the gastrointestinal tract [17]. A previous study showed that *F. nucleatum* derived AI-2 mediate the interaction between the *F. nucleatum* and periodontopathogens via biofilm formation, coaggregation and enhanced expression of adhesion molecules [18]. AI-2 also regulating macrophage infiltration into tumour microenvironments and induces CRC occurrence [19]. Our previous work showed that bacteria-derived AI-2 was associated with tumour immunity in CRC patients through inducing tumour-associated macrophages and reducing CD4/CD8 ratio in a TNFSF9-dependent manner [20]. Recently, we found that gut microbiota-derived AI-2 could

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modulate lung inflammation through the gut-lung axis [21]. Nevertheless, few studies have addressed the influence of host factors on bacterial QS [22], which remains an important area of investigation in a variety of diseases caused by microbiota imbalance, such as CRC.

It has been well recognized that a variety of hormones are secreted into the gastrointestinal tract, and influence the gastroenteric function [23,24]. Chronic stress induces norepinephrine (NE) secretion from the enteric nervous system via the brain-gut axis [25]. NE is partly produced in the enteric nervous system [26] and reaches concentrations up to 50 ng/g in the colon [27]. Gastrointestinal stress hormones can not only modulate intestinal motility, endocrine signalling, and immunity but also regulate the composition and behaviour of gut microbiota [28]. It has been reported that NE can promote growth, virulence, and QS signalling in some anaerobic bacteria [29–31]. Thus, we hypothesized that host NE might promote the progression of *F. nucleatum* related CRC involving bacterial QS system.

In the current study, we examined the abundance of *F. nucleatum* and concentrations of AI-2 and NE in 40 CRC patients at different stages. *In vitro* studies were used to investigate the role of NE in the pathogenicity of *F. nucleatum*, which was further explored in $Apc^{Min/+}$ mouse model fed with *F. nucleatum*. Our results suggest that NE promotes the progression of *F. nucleatum* related CRC, possibly through bacterial QS signalling.

Materials and methods

Clinical sample collection

Tumors and adjacent normal tissues were collected from 40 patients with CRC who underwent surgery at the Affiliated Hospital of North Sichuan Medical College (Nanchong, China). Fecal samples were collected from CRC patients before surgery and from 40 healthy volunteers. All samples were processed as previously described [32]. Clinicopathological characteristics of the samples are listed in Supplementary Table S1. CRC patients and healthy controls were age- and sex-matched. Informed consent was obtained from all participants, and the study was approved by the Institutional Review Board of the Affiliated Hospital of North Sichuan Medical College (Nanchong, China) (Approval ID: No. K2017027).

Quantification of F. nucleatum by qRT-PCR

qRT-PCR was used to calculate the relative abundance of *F. nucleatum* in tissues [33,34]. Total DNA was extracted from the tissues using the kit (DP304–03, TIANGEN, China) according to the manufacturer's instructions and was used as the template for qRT-PCR. qRT-PCR using specific primers targeting *F. nucleatum* was performed to estimate the relative abundance of *F. nucleatum*. *Gapdh* was set as a reference gene to normalize the *F. nucleatum* abundance. The relative abundance of *F. nucleatum* was calculated using the $2^{-\Delta\Delta CT}$ method [35]. Each experiment was repeated in triplicate.

Primers were listed as follows:

F. nucleatum:forward: 5'-ACCTAAGGGAGAAACAGAACCA-3;'

F. nucleatum:reverse: 5'-CCTGCCTTTAATTCATCTCCAT-3.'

Gapdh:forward: 5'-ACGGGAAGCTCACTGGCATGGCCTT-3;'

Gapdh:reverse:5'-CATGAGGTCCACCACCCTGTTGCTG-3.'

Analysis of Fusobacterium adhesin A (fadA) and luxS expression by qRT-PCR

The bacterial cultures with OD 600 = 0.7, were harvested for RNA extraction using TRIzol (R0016; Beyotime Biotechnology, China) according to the manufacturer's protocol [5]. qRT-PCR using specific primers targeting *luxS* or *fadA* were performed to estimate the level of *luxS* or *fadA*. Primers targeting *F. nucleatum*-specific 16S rRNA was set as a reference to normalize the the level of *fadA*. The relative level of *luxS* or *fadA* was calculated using the $2^{-\Delta\Delta CT}$ method [35] and normalized to the *F. nucleatum*-specific 16S rRNA. To quantify the *fadA* or *luxS* gene copies, plasmid carrying *fadA* or *luxS* was serially diluted to 10^2–10^8 *fadA* or *luxS* copies/ml and used to generate standard curves for Ct values. The *fadA* or *luxS* gene copies in the clinical samples were calculated based on the standard curves. Each experiment was repeated in triplicate [9].

Primers were listed as follows:

FadA:forward: 5'-GAAGAAAGAGCACAAGCTGA-3;'

FadA:reverse:5'- GCTTGAAGTCTTTGAGCTCT-3.'

LuxS:forward: 5'-AAGCCCCTTATGTGCGTATC-3;'

LuxS:reverse: 5' -GGATAATCTCAGCGACTAAATGC-3;'

16s rRNA:forward: 5'-ACCTAAGGGAGAAACAGAACCA-3;'

16s rRNA:reverse: 5'-CCTGCCTTTAATTCATCTCCAT-3.'

Fluorescent in situ hybridization (FISH)

FISH was performed as previously described [36]. The sequence of the FITC – labelled *F. nucleatum* - targeted probe, FUS664, was: 5′ - CGCAATACAGAGT TGAGCCCTGC – 3′. To quantify *F. nucleatum* abundance, three blind observers assessed five random fields with 200 × magnification per sample and calculated the density of *F. nucleatum*.

Bacteria culture and drug treatment

The *F. nucleatum* used in our study was separated anaerobically from a right-sided colon tumour and a single isolate *F. nucleatum* (F01) was obtained in our previous study [5]. The F01 strain was sequenced and submitted to GenBank (accession number: SUB1766768 Seq01 KX692281). The cells were cultured in fastidious anaerobic medium (FAB) supplemented with 10% foetal bovine serum in an incubator (containing 85% N_2, 10% H_2 and 5% CO_2, 37°C) (Yuejin, Shanghai, China). *Vibrio harveyi* (*V. harveyi*) BB170 was obtained from Guangdong Culture Collection Center (Guangzhou, China) and cultured at 30°C in autoinducer bioassay (AB) medium. NE and propranolol were obtained from Solarbio, China, and used to treat *F. nucleatum* at 1 ng/ml.

Bacterial growth assays

A serial dilution series of *F. nucleatum* (10^{-5}-10^{-8}) in 1 ml medium was transferred to agar plates containing 10% defibrillated sheep blood. After incubation at 37°C for 48 h in an anaerobic chamber, bacterial colonies were counted manually.

AI-2 determination

V. harveyi strain BB170 was used to detect the concentration of AI-2, as previously described [37,38]. A standard solution of dihydroxy-2, 3-pentanedione (DPD) (710374–30–4, Omm Scientific Inc., USA), an AI-2 precursor, was prepared as previously described [39]. Briefly, 10 µl of each sample was added to 90 µl of *V. harveyi* BB170 followed by incubation at 30°C for 4.5 hours in a shaker at 160 rpm. The concentration of AI-2 was calculated based on the bioluminescence value.

Mouse CRC models

All animal experiments and protocols were approved by the Animal Care and Animal Experiment Ethics Committee of the North Sichuan Medical College (Approval ID: No. 201702004). Six-week-old male C57BL/6-Apc$^{Min/+}$ mice were purchased from Jiangsu JiCui Yaokang Biotechnology Co., Ltd. and raised in an SPF environment at the Animal Experiment Center of North Sichuan Medical College. Fifty C57BL/6-Apc$^{Min/+}$ mice were randomly assigned into five groups: (1) Control; (2) *F. nucleatum*; (3) *F. nucleatum* + D-Ribose (D-Rib); (4) *F. nucleatum* + Stress; (5) *F. nucleatum* + Stress + α-methyl-p-tyrosine (AMPT). The C57BL/6-ApcMin/+ mice were fed by gavage with *F. nucleatum* at approximately 109 colony-forming units (cfu) of *F. nucleatum* in FAB medium daily for 8 consecutive weeks. D-Rib, an AI-2 inhibitor by competitively binding with the AI-2 receptor RbsB [40], was diluted in drinking water (100 mM final concentration) and replaced every other day. On Day 40 of *F. nucleatum* administration, mice were subjected to a chronic stress paradigm. For the chronic stress model, mice administered *F. nucleatum* for 40 days daily were placed in a well-ventilated 50 mL centrifuge tube for 2 h per day for 20 consecutive days [41]. Contemporaneously, AMPT (OKA, China, 100 mg/kg) was administered by intraperitoneal injection for 20 consecutive days.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to detect the concentration of NE in human and mouse tumours [42,43]. Intestinal tumour tissues were mixed with PBS at a ratio of 1:9 (w/v). After homogenization and centrifugation (4°C, 5000 rpm, 20 min), the supernatant was collected and stored at −20°C. To determine the concentration of NE, 20 µl supernatant was subjected to mouse or human NE ELISA kit (Andygen, U.S. AD11667Hu or AD3383Mo). A standard curve was generated using the reference standard set provided by the kit. The samples were measured according to the manufacturer's instructions accompanying the kit. The NE concentration was calculated using a standard curve.

Transcriptome sequencing

The total RNA was extracted from *F. nucleatum* with/without NE treatment. The mRNA was purified from total RNA for library preparation. The mRNA was randomly fragmented and used as the template for the synthesis of first strand cDNA. The second strand cDNA was synthesized with dNTP that containing dUTP instead of dTTP in the DNA

polymerase I system. After adenylation and adaptor ligation, the second strand cDNA was fragmented, followed by purification with AMPure XP system (Beckman Coulter, Beverly, USA) to obtain fragments of 370 ~ 420 bp in length. Then these fragments were amplified by PCR and purified for quality control on the Agilent Bioanalyzer 2100. Next, the qualified index-coded samples were clustered using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) according to the provided protocol. At last, the library sequencing was performed on an Illumina Novaseq platform and the paired-end reads of 150 bp were generated. For the transcriptome sequencing analysis, the data were blasted with the reference sequence (NZ_CP053468.1). The gene transcription level was evaluated with the RSEM package, and the up- or down-regulated genes were selected for functional analysis.

Results

F. nucleatum and AI-2 accumulated with the progression of CRC

We used Fluorescent in Situ Hybridization (FISH) analysis to label *F. nucleatum* in the tumours and adjacent normal tissues of patients with CRC. Consistent with previous findings, we observed an accumulation of *F. nucleatum* in tumour tissue and less accumulation in nearby non-neoplastic tissues (Figure 1a). To quantify the abundance of *F. nucleatum* in each sample, qRT-PCR analysis was performed, and the results were consistent with the FISH data ($p < 0.0001$) (Figure 1b). Furthermore, we noted that the abundance of *F. nucleatum* was higher in advanced stage 4 tumours than in the early clinical stages (stages 1 and 2, $p < 0.01$) (Figure 1c), suggesting that *F. nucleatum* may be correlated with CRC progression.

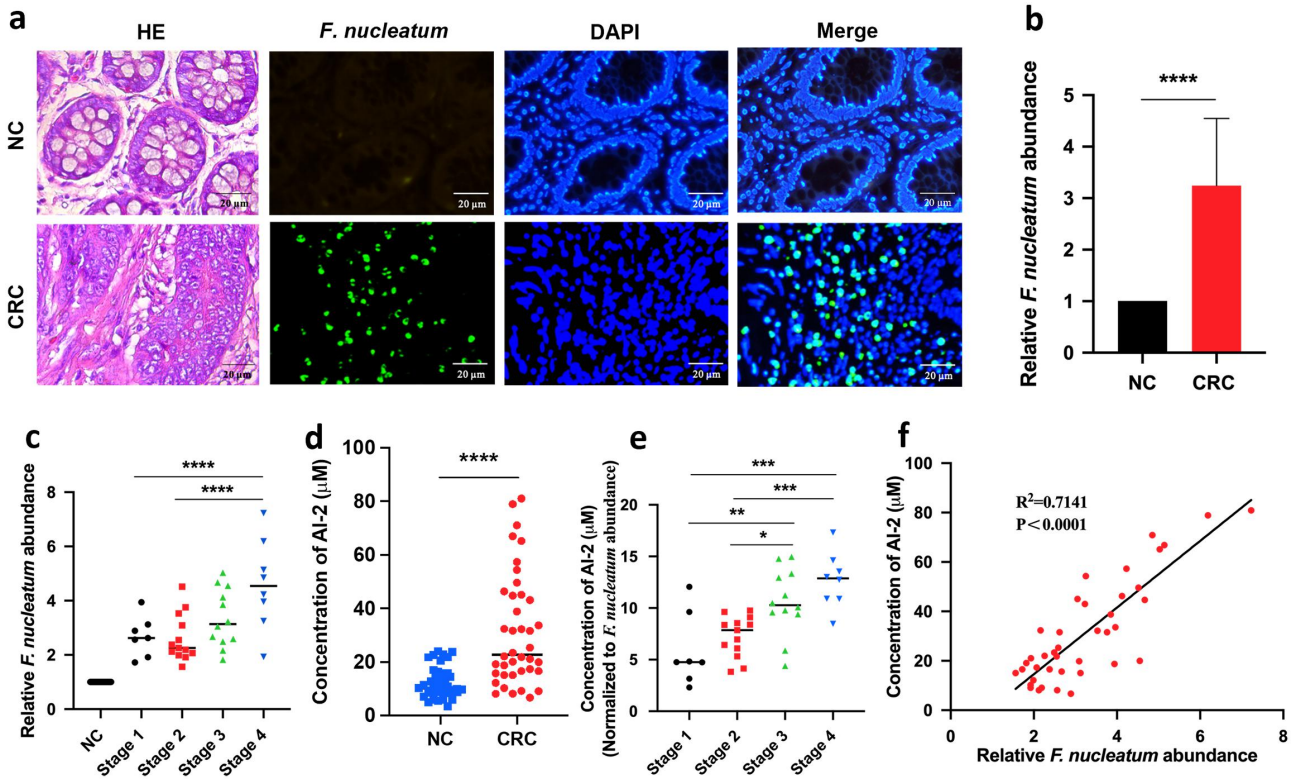

Figure 1. *F. nucleatum* increased along with CRC progression and AI-2 accumulation (a) Representative images of FISH detecting *F. nucleatum* in CRC and NC tissues. (b) qRT-PCR data showed the significantly increased abundance of *F. nucleatum* in CRC tissues ($n = 40$) compared with that in NC tissues ($n = 40$). The relative abundance of *F. nucleatum* in tissues was estimated by qRT-PCR using the specific primers targeting *F. nucleatum*, and primers targeting *gapdh* were used to confirm the equal quantity of tissues. (c) qRT-PCR data showed the increased abundance of *F. nucleatum* along with the cancer progression. (d) the concentration of AI-2 is significantly higher in CRC tissues compared with NC tissues, and (e) increased along with the cancer progression. (f) Pearson's correlation analysis showed a positive correlation between *F. nucleatum* abundance and AI-2 concentration in tissues of CRC patients.

****$p < 0.0001$, **$p < 0.01$. *$p < 0.05$. *F. nucleatum*, *Fusobacterium nucleatum*; CRC, colorectal cancer; AI-2, auto-inducer-2; FISH, Fluorescent in Situ Hybridization; NC, adjacent normal control.

Next, we evaluated the concentration of AI-2, a major molecule in the bacterial QS system. Concomitant with increased *F. nucleatum* abundance in tumour tissue, the concentration of AI-2 in tumour tissue was significantly higher than that in adjacent normal tissue ($p < 0.0001$) (Figure 1d) and continued to increase along with the clinical stage of cancer (Figure 1e). In addition, AI-2 concentration was significantly higher in stools from CRC patients ($n = 40$) than in healthy volunteers ($n = 40$, $p < 0.0001$) (Fig. S1A) and increased with CRC progression (Fig. S1B). Notably, Pearson's correlation analysis showed a positive correlation between *F. nucleatum* abundance and AI-2 concentration in CRC tumour samples ($p < 0.0001$) (Figure 1f).

Taken together, these results suggest that the bacterial QS molecule AI-2 May be associated with the progression of *F. nucleatum* related CRC.

NE increased with the progression of F. nucleatum *related CRC*

We determined the concentrations of NE in both tumour and adjacent normal tissues using ELISA. The concentration of NE was significantly higher in the tumours ($n = 40$) than in the adjacent normal tissues ($n = 40$, $p < 0.0001$) (Figure 2a).

Notably, the concentration of NE increased with CRC progression (Figure 2b). Additionally, we found a positive correlation between NE concentration and *F. nucleatum* abundance (Pearson's correlation, $r = 0.5038$; $p < 0.0001$), as well as between NE concentration and AI-2 concentration (Pearson's correlation, $r = 0.7616$; $p < 0.0001$) (Figure 2c,d). These results suggest that host-derived NE may be associated with the progression of *F. nucleatum* related CRC, possibly involving bacterial AI-2 signalling.

NE promoted the virulence and proliferation of F. nucleatum *with upregulation of QS system*

To study the role of NE in *F. nucleatum* activity, we analysed the gene expression profile of *F. nucleatum* after NE treatment for 24 h. NE treatment induced a QS system that utilized autoinducers (AIs) (Figure 3a). The QS-associated genes *ccfA*, *ABC.PE.S*, and, to a lesser extent, *oppA*, *blcC*, and *crp* were among

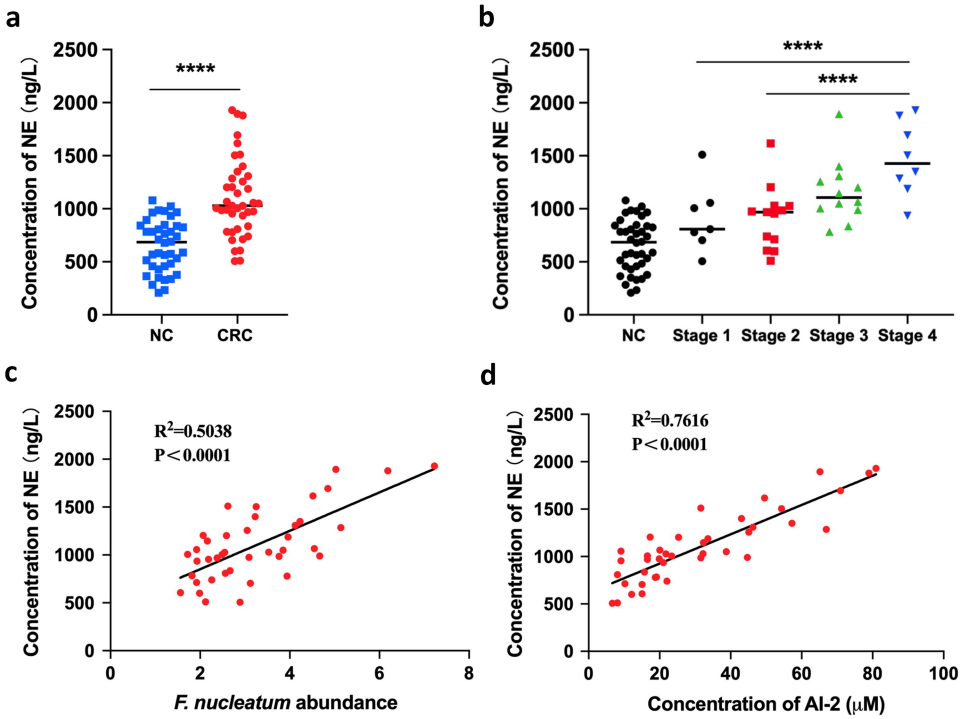

Figure 2. NE increased with the progression of *F. nucleatum* related CRC (a) the concentration of NE was significantly higher in CRC tissues ($n = 40$) compared with NC tissues ($n = 40$). (b) the concentration of NE in tumours increased along with the progression of CRC. (c, d) Pearson's correlation analysis showed positive correlation between NE concentration and *F. nucleatum* abundance (c) or AI-2 concentration (d) in CRC tissues.

*****P* < 0.0001, ***P* < 0.01. CRC, colorectal cancer; NC, adjacent normal control; NE, norepinephrine; *F. nucleatum*, *Fusobacterium nucleatum*; AI-2, auto-inducer-2.

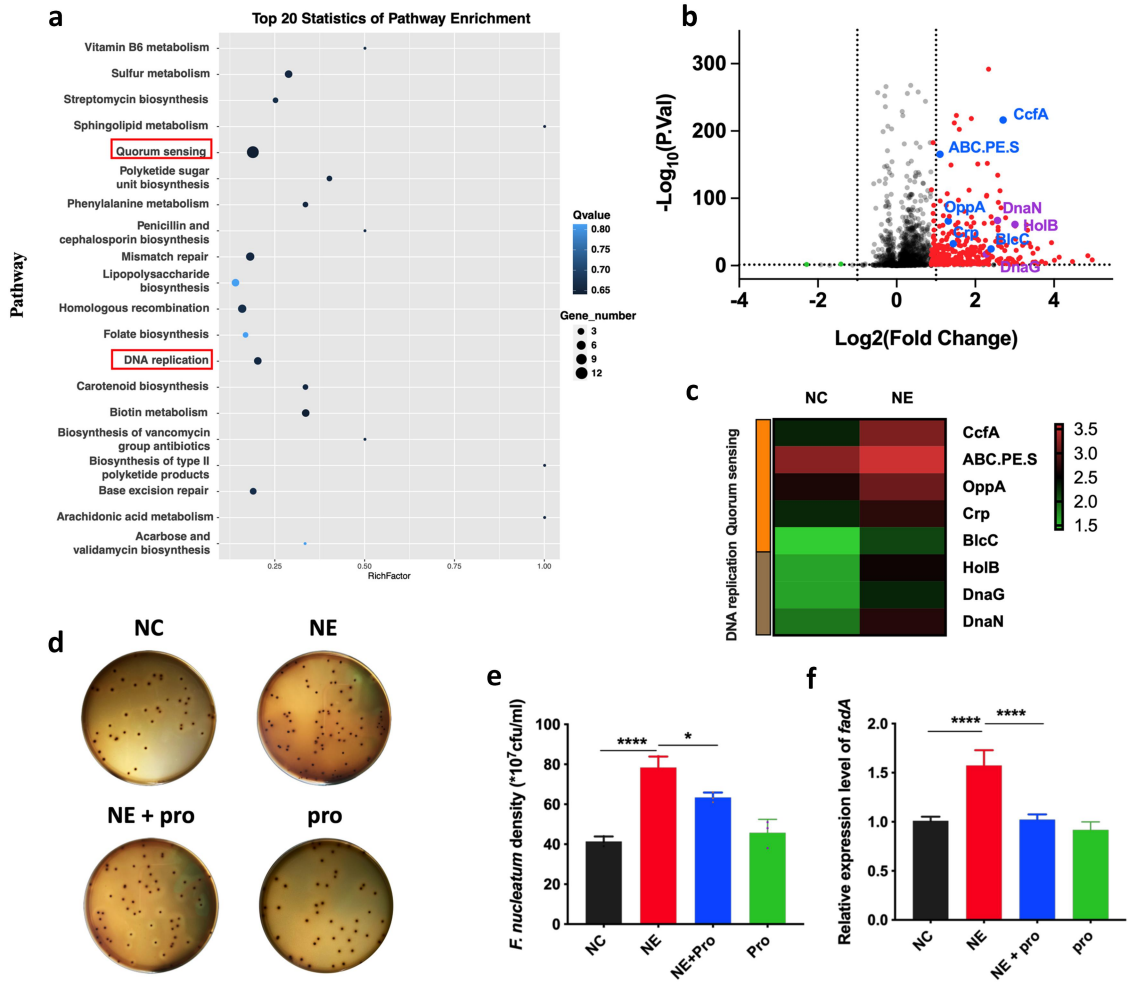

Figure 3. NE promoted the virulence and proliferation of *F. nucleatum* with upregulation of QS system (a) *F. nucleatum* was treated with NE for 24 hours followed by subjection to RNA-Seq. KEGG analysis of pathway enrichment showed the induction of QS after NE treatment. (b) volcano plot of the transcriptome in NE group versus NC group. QS associated genes (*ccfA*, *ABC.PE.S*, *oppA*, *crp*, *blcC*) and DNA replication associated genes (*holB*, *dnaG*, *dnaN*) were labelled. (c) Heatmap of differentially regulated genes associated with QS and DNA replication. (d) Representative images of the colony formation of *F. nucleatum* subjected to NE and propranolol treatments. (e) statistics of *F. nucleatum* density based on the colonies in (d). (f) qRT-PCR data showed that *fadA* expression was increased significantly with treatment of NE, and decreased by propranolol. All experiments were repeated three times, and one-way analysis of variance was used.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. *F. nucleatum*, *Fusobacterium nucleatum*; QS, quorum sensing; NC, Negative control; NE, norepinephrine; pro, propranolol, NE inhibitor.

the upregulated genes in the volcano plot and heatmap (Figure 3b,c), which are consistent with previous studies [44–47]. Moreover, DNA replication was observed in the transcriptomic analysis, which may suggest proliferation of *F. nucleatum* after NE treatment (Figure 3a).

Therefore, we tested whether NE affected the growth and virulence of *F. nucleatum*. Using a colony formation assay, we found that NE promoted *F. nucleatum* proliferation (Figure 3d,e), whereas propranolol, an NE inhibitor, reversed the growth rate ($p < 0.05$) (Figure 3d,e). *FadA* of *F. nucleatum*, a virulence factor that regulates cell adhesion and invasive properties, was upregulated in response to NE treatment (Fig. S2), whereas its inhibitor decreased *fadA* expression ($p < 0.05$) (Figure 3f). These findings suggest that host NE may enhance the growth and virulence of *F. nucleatum* possibly through bacterial QS signalling.

AI-2 is essential for NE enhanced virulence and proliferation of F. nucleatum

In addition, the level of AI-2 was significantly increased by NE ($p < 0.05$) (Figure 4a). Next, we treated *F. nucleatum* with D-Rib, an AI-2 competitive inhibitor, in combination with NE. The results showed that

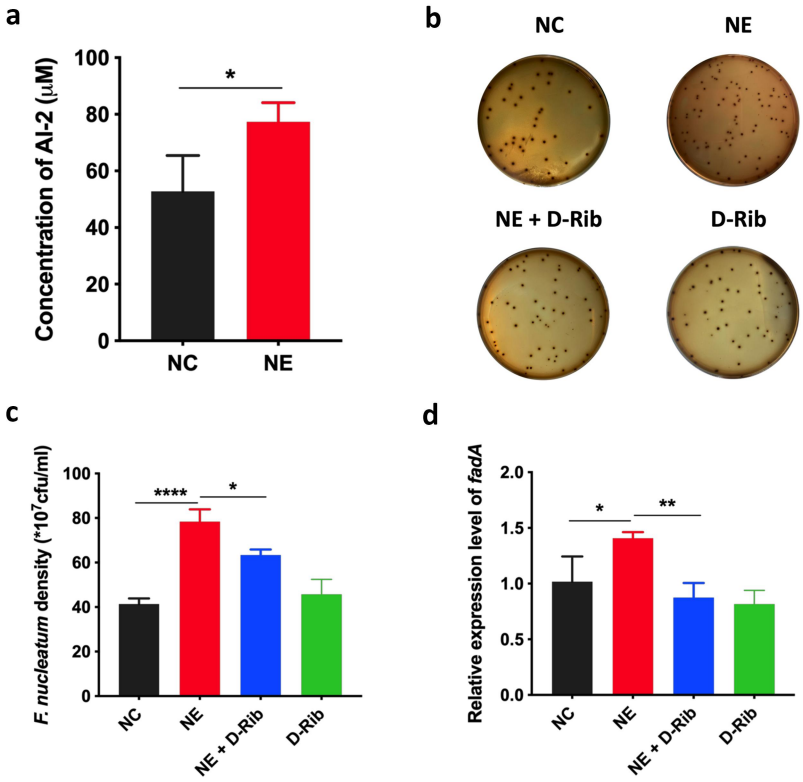

Figure 4. AI-2 is essential for NE enhanced virulence and proliferation of *F. nucleatum* *(a) statistics of AI-2 concentration in the supernatant of F. nucleatum culture induced by NE. (b) Representative images illustrating the colony formation of F. nucleatum subjected to NE treatments with and without AI-2 inhibitor. (c) statistics of F. nucleatum density based on the colonies in (b). (d) qRT-PCR data showed that D-Rib significantly inhibited the FadA expression of F. nucleatum induced by NE. All experiments were repeated three times, and paired t test was used.*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. *F. nucleatum*, *Fusobacterium nucleatum*; NC, Negative control; NE, norepinephrine; D-Rib, D-Ribose; AI-2 competitive inhibitor.

D-Rib inhibited the growth of *F. nucleatum* induced by NE ($p < 0.05$) (Figure 4b,c). Moreover, D-Rib significantly inhibited *fadA* expression of *F. nucleatum* induced by NE in F. nucleatum ($p < 0.05$) (Figure 4d). These results suggest that AI-2 signalling is required for the growth and virulence of *F. nucleatum* induced by NE.

Stress accelerated the progression of colorectal tumor in Apc$^{Min/+}$ mice infected with F. nucleatum

Our previous studies have demonstrated that *F. nucleatum* administration can potentiate colorectal tumorigenesis in mice [48], and that AI-2 concentration was increased in both colorectal tissue and stool of CRC patients [20]. To investigate the role of AI-2 in *F. nucleatum* related CRC *in vivo*, we treated *F. nucleatum* fed Apc$^{Min/+}$ mice with D-Rib, an AI-2 inhibitor. The tumour number and size in the intestine were lower in D-Rib-treated mice than in *F. nucleatum* group ($p < 0.01$, $p < 0.05$, respectively), suggesting that AI-2 signalling is required to enhance *F. nucleatum* induced colorectal tumours *in vivo* (Figure 5a,b,).

To investigate the role of NE in *F. nucleatum* related colorectal tumours *in vivo*, we used a mouse model in which NE production was induced by subjecting the mice to chronic stress. The concentration of NE significantly increased in the tumours of mice subjected to stress ($p < 0.05$), which was counteracted by AMPT treatment ($p < 0.01$) (Fig. S3). Based on *F. nucleatum* administration, chronic stress accelerated intestinal tumour formation, with increased tumour number (although not significant) and size ($p < 0.01$) (Figure 5a,b,). Moreover, both the tumour number and size were significantly decreased by the NE inhibitor AMPT ($p < 0.05$) (Figure 5a,b). Additionally, AMPT significantly decreased the abundance of *F. nucleatum* induced by host stress ($p < 0.001$) (Figure 5d). In this mouse model, we also evaluated the concentration of AI-2, showing that *F. nucleatum* fed mice showed higher AI-2 level, which was rescued by D-Rib or AMPT (Figure 5e).

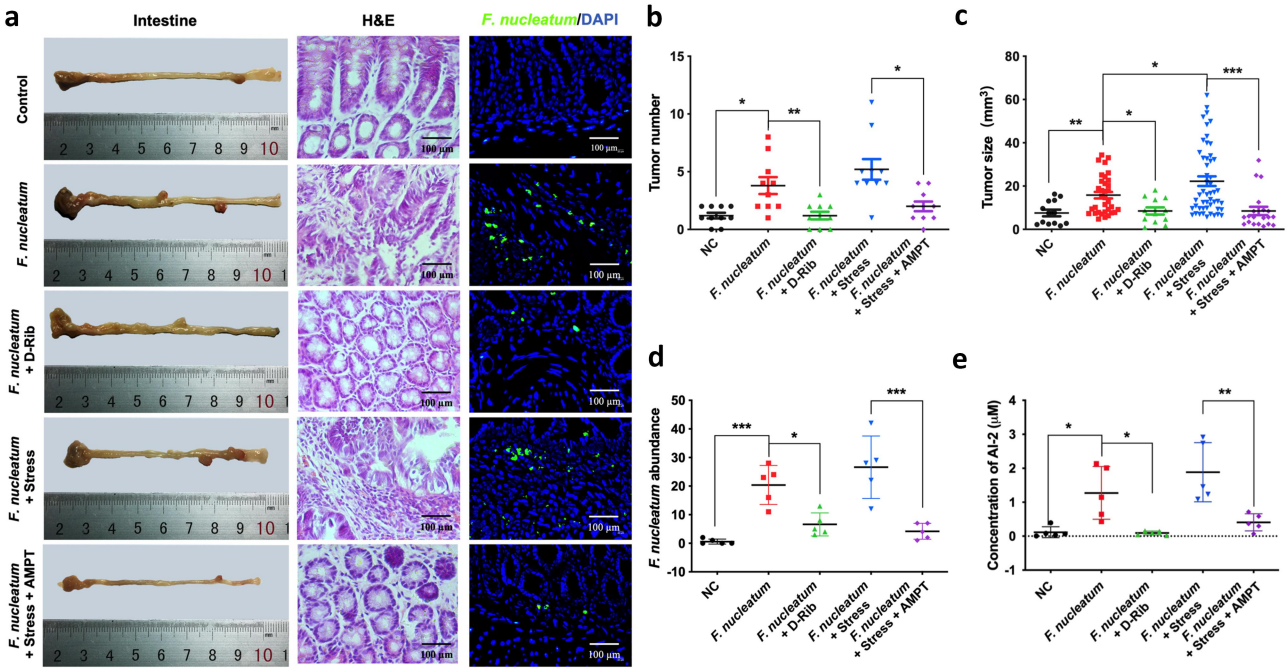

Figure 5. Stress promoted the progression of colorectal tumour in $Apc^{Min/+}$ mice fed with *F. nucleatum (a) Representative images showing the tumor formation and F. nucleatum invasion in tumours. (b, c) quantification of tumour number and size in each group. D-Rib significantly decreased the tumour number and size in mice fed with F. nucleatum. the tumour size in F. nucleatum + stress group was significantly higher than in F. nucleatum group. Both the tumour number and size were decreased significantly by catecholamine inhibitor AMPT. (d) qRT-PCR analysis highlighting F. nucleatum burden in tumours. AMPT significantly decreased the abundance of F. nucleatum induced by host stress. (e) F. nucleatum administration significantly increased AI-2 level in tumours, which was inhibited by D-Rib or AMPT.*

****P* < 0.001, ***P* < 0.01. **P* < 0.05. *F. nucleatum*, *Fusobacterium nucleatum*; D-Rib, D-Ribose; AMPT, catecholamine inhibitor; NC, Negative control.

Discussion

Previous studies have reported that *F. nucleatum* is enriched in tumour tissues and stools of CRC patients and is positively correlated with tumour stage [3]. However, as an opportunistic pathogen, *F. nucleatum* also exists in the healthy gut, raising the question of how *F. nucleatum* pathogenicity in CRC is regulated by host factors. In the present study, we found that the stress hormone NE can support the proliferation and virulence of *F. nucleatum in vitro* and mainly drive an increase in tumour size in $APC^{Min/+}$ mice fed *F. nucleatum*, which was counteracted by the catecholamine inhibitor AMPT *in vivo* and *in vitro*. These results suggest that the host stress hormone NE promotes the progression of *F. nucleatum* related CRC.

It has been shown that *F. nucleatum* adhere to and invade epithelial cells via virulence factors, including *fadA*, Fusobacterium autotransporter protein 2 (Fap2), and Fusobacterial outer membrane protein A (FomA) [9,49–51]. Here, we found that NE promoted the growth of *F. nucleatum in vitro* and induced *fadA* expression. These effects were inhibited by an AI-2 inhibitor, suggesting that the pathogenicity of *F. nucleatum* is regulated by QS signalling.

Although it has been reported that stress promotes CRC progression [52], the underlying mechanism remains to be elucidated, especially in *F. nucleatum* related CRCs. Our results revealed a positive correlation between NE concentrations and AI-2 levels in human CRC tissues. We further analysed the gene expression profile of *F. nucleatum* after NE treatment and found that QS-associated genes, such as *ccfA* and *ABC. PE. S*, were upregulated. These interesting findings indicate, for the first time, that host stress may promote the progression of *F. nucleatum* related CRC through NE/AI-2 signalling.

To our knowledge, this is the first study to address the mechanism of *F. nucleatum* related CRC with a focus on the brain-gut axis and the inter-kingdom signalling communication between the host neuroendocrine system and bacteria. Our results suggest a new strategy for the management of *F. nucleatum* related CRC by targeting inter-kingdom signalling between host stress hormones and the bacterial QS system.

This strategy may also benefit other diseases associated with brain-gut axis dysfunction or gut microbiota dysbiosis, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethics statement

This study was approved by the Institutional Review Board of the Affiliated Hospital of North Sichuan Medical College (Nanchong, China), and informed consent was obtained from all participants prior to enrolment (Approval ID: No. K2017027).

All animal experiments and protocols were approved by the Animal Care and Animal Experiment Ethics Committee of the North Sichuan Medical College (Approval ID: No. 201702004). Six-week-old male C57BL/6-Apc$^{Min/+}$ mice were purchased from Jiangsu JiCui Yaokang Biotechnology Co., Ltd. and raised in an SPF environment at the Animal Experiment Center of North Sichuan Medical College.

Consent for publication

The manuscript is approved by all the participants.

Author contributions

The work reported in this paper was performed by the authors unless clearly specified in the text. XHD, ZZT, and LY conducted the experiments and analysed the data. XHD, ZZT, LY and MZ drafted the manuscript. MZ and XSF refined and approved the final manuscript. XSF conceived and designed the study. Other co-authors performed helpful work. All authors have contributed to the manuscript and approved the submitted version.

Data Availability statement

The authors confirm that the data supporting the findings of this study are available in the article and its supplementary materials.

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