MicroRNA-125a-3p participates in odontoblastic differentiation of dental pulp stem cells by targeting Fyn

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Abstract Fyn is a member of the protein tyrosine kinase family and its overexpression is associated with various types of inflammation. MicroRNAs can regulate the expression of target genes and play an important role in varied physiological and pathological processes. Based on the important role of Fyn and microRNA-125a-3p (miR-125a-3p) in inflammation, and combined with the bioinformatics studies, we performed in this study and chose miR-125a-3p as the focus of our research. During the progression of inflammation, we found that the expression of miR-125a-3p was decreased while the expression of Fyn

was up-regulated. Fyn formed a complex with Neuropilin-1, which inhibited odontoblastic differentiation and expanded inflammatory responses through nuclear factor- κ B signal pathways in dental pulp stem cells (DPSCs). These findings suggested that miR-125a-3p plays an important role in odontoblastic differentiation of DPSCs by targeting Fyn, implying its therapeutic potential in dental caries.

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Introduction

Deep caries refer to the progression of caries to the deep dentin, which causes the repair of dental pulp tissue, including the formation of dentin, mild chronic inflammatory reaction, vasodilatation, odontoblast disorder, and so on. When the injury reaches the pulp, it can cause pulpitis, and further development will cause periapical abscess, leading to osteomyelitis of the jaw (Schmalz and Galler 2011). Many studies showed that bacteria stimulate the production of proinflammatory cytokines, which is closely related to the occurrence and development of dental caries (Evans et al. 1990; Gornowicz et al. 2012; Kim et al. 2002; Soell et al. 1994). Under inflammatory stimulation, dental pulp stem cells (DPSCs) produced reparative dentin to maintain the integrity of the tooth structure. The destruction of odontogenic teeth was faster than the formation of teeth, eventually leading to dental caries (Larmas and Sandor 2014). DPSCs are self-renewal multipotent stem cells that can differentiate into the mature phenotype of the mesenchymal germ layer. Their morphology and function are similar to those of mesenchymal stem cells (Gronthos et al. 2000). The most notable feature of DPSCs is the ability to form dentin-pulp-like complex (Alge et al. 2010; Gronthos et al. 2002). Our group's previous found that NRP1-Fyn compound excited NF- κ B signaling pathway to restrain the odontoblast differentiation of DPSCs (Song et al. 2018). In recent years, the discovery of post transcriptional regulation of microRNAs (miRNAs) has attracted widespread attention. But so far, it has not been reported whether miRNAs participate in the development of dental caries.

Fyn, a member of the protein tyrosine kinase Src family, is one of the important receptors of cell surface receptors and it is widely expressed in human tissues. Activation of Fyn is involved in cell proliferation, apoptosis, adhesion, movement, cytoskeleton reconstruction and many other important physiological processes (Alge et al. 2010; Elias and Ditzel 2015). Abnormal expression of Fyn could be detected in a

variety of pathological processes, such as Alzheimer's disease and the progression of many types of cancer, including epithelial to mesenchymal transition and metastasis (Lewin et al. 2010; Rivera and Olivera 2007). Studies showed that Fyn is involved in the imbalance of inflammation and autoimmune diseases, the detection of Fyn activation markers in patients with lupus nephritis highlights the importance of Fyn balance (Mkaddem et al. 2017). Neuropilin-1 (NRP1) is one of the members of neuropilin family. Previous studies showed that NRP1 interacting with Fyn is involved in the process of odontoblastic differentiation of DPSC and thus plays a role in the development of caries (Song et al. 2017, 2018). Owing to its numerous functions and participate in NF- κ B signaling pathways, we anticipated that the expression and activity of Fyn should be strictly regulated.

MicroRNAs (miRNAs) are a class of non-coding RNA molecules composed of 20–23 nucleotides that regulate the expression of target gene by combining with the target gene's 3' untranslated region (3'UTR) (Bartel 2004). More and more evidence suggested that miRNAs may be involved in the development and/or maintenance of inflammation and neuropathic pain (Dong et al. 2014). It was reported that miR-125a-3p has a negative correlation with the development and maintenance of oral inflammatory pain by regulating p38 and MAPK genes (Dong et al. 2014). According to the prediction website, we found that miR-125a-3p was closely related to the regulation of Fyn. It was reported that miR-125a-3p regulates Fyn mRNA and its protein expression level, suggested that miR-125a-3p can be used as a therapeutic agent for related disorders caused by abnormal Fyn (Ninio-Many et al. 2013). It was found that miR-125a-3p regulates the expression of GIT1 to inhibit osteoblastic proliferation and differentiation (Tu et al. 2016).

In this study, we aimed to analysis the role of miRNA in the odontoblastic differentiation of human DPSCs and to explore its role in the development of dental caries. Our study showed that miR-125a-3p regulated Fyn imbalance in the inflammation model, resulting the expression of Fyn decreased in the odontoblastic differentiation of DPSCs. It meant that miR-125a-3p play an important role in regulating the expression of Fyn and its signaling pathway, thus had therapeutic potential in deep caries.

Materials and methods

Cell cultures

We selected five healthy men and five men with deep caries in the stomatology department of Nantong University Affiliated Hospital. The deep caries diagnosis was based on clinical evaluation by the doctor of endodontology. All participants had no periodontal infections, root lesions and no history of systemic disease. They rinsed with sodium bicarbonate solution before tooth extraction and signed informed consent for tissue collection and usage. The protocol was approved by the Ethical Committee of Nantong University Affiliated Hospital. DPSCs were isolated and cultivated according to the methods used by our group before (Song et al. 2017, 2018). Briefly, in a sterile environment, teeth were cut using a dental slit needle to expose the medullary cavity. Pulp tissues were digested with 3 mg/mL collagenase solution at 37 °C for 1 h, and single cell suspensions were obtained by a 70 µm cell strainer (BD Falcon) and inoculated in high glucose Dulbecco's modified Eagle's medium (H-DMEM) containing 10% Fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL streptomycin (Ledesma-Martinez et al. 2016).

Our research team verified that the cells were dental pulp stem cells (DPSCs) (Feng et al. 2013). The cells derived from healthy men were named N-DPSC, and the cells derived from patients with deep caries were named I-DPSC. DPSCs were cultured for 3 to 5 passages for this experiment.

Odontoblastic differentiation

DPSCs (2×10^4 cells/dish) were cultured in 35 mm culture dishes (Costar, Cambridge, MA) in odontoblastic differentiation medium containing a minimum essential medium (Invitrogen, Carlsbad, CA), 15% FBS (Gibco-BRL; Life Technologies, Inc., Gaithersburg, MD), 10 mmol/L β-glycerophosphate, 50 mg/mL α-ascorbic acid, 10 nmol/L dexamethasone (Sigma-Aldrich, St Louis, MO), 0.292 mg/mL glutamine, 100 mg/mL streptomycin, and 100 U/mL penicillin G for 21 days, replacing the medium every 2 days.

Alizarin red staining and alkaline phosphatase (ALP) staining

The DPSCs were placed in odontoblastic differentiation induction medium containing 0.1 µM dexamethasone, 10 mM β-glycerophosphate (Sigma) and 50 µg/mL ascorbic acid (Sigma). DPSCs were incubated in the absence or presence of TNF-α (50 ng/mL) for 14 days. The degree of extracellular matrix calcification was assessed by staining with Alizarin Red S (Sigma) and alkaline phosphatase (Jian Cheng, Nanjing, China).

The DPSCs were fixed with 4% PFA for 1 h and then washed with PBS, stained with 40 mmol/L Alizarin Red S (pH = 4.2) for 10 min, and the absorbance of the extracted alizarin red S stain was measured at 570 nm. ALP staining was performed according to the ALP (Jian Cheng, Nanjing, China) assay kit instructions.

Western blot analysis

The collected DPSCs were soaked with the configured buffer (protease inhibitor and cell lysate 100:1) and incubated on ice for 30 min. Centrifuge at 12,000 rpm for 10 min. The protein content of the collected supernatant was quantified by the BCA kit (Beyotime, Shanghai, China). The proteins were subjected to SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto polyvinylidene fluoride membranes at 300 mA for 1.5 h in a blotting apparatus (BioRAD, CA). After blocking in Tris-buffered saline with Tween with 5% nonfat dry milk, the membranes were incubated overnight at 4 °C with primary antibodies. Then, membranes were reacted with corresponding horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 h. Use GADPH (anti-mouse; Santa Cruz) as an internal reference, other primary antibodies are: Neuropilin-1 (NRP1; anti-rabbit; Santa Cruz), Fyn (anti-mouse; Santa Cruz), dentin matrix protein-1 (DMP1; anti-rabbit; Santa Cruz), dentin sialophosphoprotein (DSPP; anti-rabbit; Santa Cruz).

Immunofluorescent staining

DPSCs were planted with a density of 1×10^5 cells/mL. After 24 h of cell attachment, DPSCs were fixed by 4% paraformaldehyde was fixed for 1 h. The

primary antibody was diluted in proportion with Immunostaining Blocking/Primary Antibody Dilution Buffer (Sangon Biotech) and incubated overnight at 4 °C. DPSCs were incubated with the corresponding secondary antibody for 2 h at room temperature. Nuclei were stained with DAPI (1:1000; Santa Cruz) and the cells were mounted on an inverted fluorescent microscope and photographed.

Quantificational real-time polymerase chain reaction (qRT-PCR)

Total cellular RNA was extracted from the cells and reverse transcribed using conventional protocols. We used the following primer sequence (Generay, Shanghai, China) for amplification: GAPDH, forward: 5'-TCCATGACAACCTTGGTATCG-3' and reverse: 5'-TGTAGCCAAATTCGTTGTCA-3'; NR1, forward: 5'-TGAGCCCTGTGGTTTATTCC-3' and reverse: 5'-CGTACTCCTCTGGCTTCTGG-3'; Fyn, forward: 5'-CTCAGCACTA-CCCCAGCTTC-3' and reverse: 5'-ATCTC CTTCCGAGCTGTTCA-3'; DMP1, forward: 5'-TGGG GATTATCCTGTGCTCT-3' and reverse: 5'-GCTGTCACT GGGGTCTTCA T- 3'; DSPP, forward: 5'-GGAGACAAGACCTCCAA-GAGTA-3' and reverse: 5'-TGCTGGGACCCTTGATTCTA-3'; Human-miR-125a-5p, forward: 5'-CGGGCTCCCTGAGACCCTTTAA-3', reverse: 5'-CAGCCACAAAAGAGCACAAAT-3' and Stem ring sequence: CCTGTTGTCTCCAGCCACAAAA GAGCACAATATTTTCAGGAGACAACAGGTCAC AGG; Homo U6, forward: 5'-CTCGCTTCGGCAG CACA-3' and reverse: 5'- AACGCTTCAC-GAATTTGCGT-3'. GAPDH and U6 were used as the reference internal control. Three replicates were set up three times for each experiment.

MicroRNA (miRNA) selection and transfection

In this experiment, we first searched for miRNA associated with Fyn and inflammation, and screened miR-125a-3p. The binding sites of miR-125a-3p and Fyn were discovered through prediction sites TargetScan, miRbase, microRNA.org and starBase V2.0.

The miR-125a-3p control, mimic and inhibitor (Biomics Biotech, Nanjing, China) were transfected into DPSCs according to the instructions of Lipofectamine 2000 (Thermo, MA, USA). According to preliminary experiments, the final concentrations of

mimic and Inhibitor transfection were 20 nM and 100 nM, respectively, and the efficiency of transfection was verified by qRT-PCR.

Cell viability assay

DPSCs were seeded on 96-well plates at a cell density of 1×10^3 cells/well. 0, 10, 20, 50, 100 ng/mL tumour necrosis factor- α (TNF- α) were add into odontoblastic inducing medium and were used to culture DPSCs for 21 days. Then we washed the wells, added 100 μ L H-DMEM and 10 μ L CCK8 (Beyotime, Shanghai, China) per well, and incubated the cells at 37 °C for 2 h. The optical density was measured down at 450 nm.

Enzyme-linked immunosorbent assay (ELISA)

After stimulating cells with different concentrations of TNF- α , the supernatant was collected, and interleukin-6 (IL-6) and interleukin-8 (IL-8) expression levels were measured according to the manufacturer's protocol of Human IL-6 ELISA Assay kit (BD Biosciences, SanJose, CA, USA) and Human IL-8 ELISA Assay kit (R&D systems, Minneapolis, MN, USA).

Statistical analysis

We used GraphPad Prism software to analyze this experimental data. All experiments were repeated at least three times, each time three replicates were designed, and the data showed mean—standard deviation. Statistical significance was assessed by independent sample t test. $P < 0.05$ was considered statistically significant.

Results

The expression of Fyn was increased in DPSCs in the microenvironment of dental caries

In order to prove whether the expression of Fyn in DPSCs separated from dental caries (I-DPSCs) is different from the expression of Fyn in DPSCs from normal tissue (N-DPSCs), we collected normal and deep caries tissues, isolated and cultured DPSCs respectively. We found that there is no significant difference in morphology between N-DPSCs and

I-DPSCs (Fig. 1a). There was no significant difference in proliferation and cell viability between the two groups (Fig. 1b). By Western blot (WB) and Quantificational real-time polymerase chain reaction (qRT-PCR), we demonstrated that Fyn is highly expressed in I-DPSCs (Fig. 1c, d). In our previous study, we found that the interaction between NRP1 and Fyn has an effect on the odontoblastic differentiation of DPSCs (Ledesma-Martinez et al. 2016). These data indicated that abnormal expression of Fyn was associated with inflammation and affected the differentiation of DPSCs into osteogenic dentin.

In vitro simulation of deep caries microenvironment

Since I-DPSCs cultured in a normal local microenvironment may influence its biological characteristics, we intended to model the inflammation microenvironment through tumor necrosis factor- α (TNF- α). We used different concentrations of TNF- α (0, 10, 20, 50, 100 ng/mL) to stimulate N-DPSCs, and found no

significant difference between 0, 10, 20 and 50 ng/mL TNF- α concentrations (Fig. 2a). After stimulation with TNF- α , DPSCs were incubated with normal medium for 24 h. The cell supernatants were harvested, interleukin-6 (IL-6) and interleukin-8 (IL-8) were detected by Enzyme-linked immunosorbent assay (ELISA). We found that at 10 ng/mL TNF- α stimulated concentration, the levels of IL-6 and IL-8 were the lowest, with the concentration increased, the levels of IL-6 and IL-8 gradually increased (Fig. 2b). The expression of TNF- α mRNA was detected by qRT-PCR, and the results were consistent with those in ELISA (Fig. 2c). Considering the cell viability and the release of inflammatory factors after stimulation with different concentrations of TNF- α , we chose 50 ng/mL TNF- α as the stimulation concentration in this study. Under 50 ng/mL TNF- α stimulated concentration, we found that the expression of Fyn is higher than unstimulated group (Fig. 2d, e). Our previous studies had confirmed that the odontoblastic differentiation of DPSCs was decreased in deep caries. Through the analysis of alizarin red and ALP staining,

Fig. 1 The expression of Fyn was increased in DPSCs in the microenvironment of dental caries. **a** The morphology of N-DPSC, I-DPSC were observed and pictured by an inverted microscope (magnification $\times 200$). **b** The cell viability of N-DPSCs and I-DPSCs were assayed and compared by CCK8. **c** The protein expression of Fyn in N-DPSC and I-DPSC was

analyzed by Western blot (WB) and quantitative analysis of WB. **d** Differences in Fyn mRNA expression in N-DPSC and I-DPSC were detected by qRT-PCR. **e** Immunofluorescence observation of Fyn distribution in N-DPSC and I-DPSC were collected. Blue is DAPI and represents the nucleus; green is Fyn (magnification $\times 200$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Fig. 2 In vitro simulation of deep caries microenvironment. DPSCs were cultured in odontogenic differentiation medium containing different concentrations of TNF- α (0, 10, 20, 50, 100 ng/mL) for 21 days. **a** The cell viability of DPSCs at different stimulating concentrations was detected by CCK8. **b** The contents of interleukin-6 (IL-6) and interleukin-8 (IL-8) in cell supernatant were detected by ELISA. **c** TNF- α mRNA expression level in DPSCs was detected by qRT-PCR. **d** WB

analysis of Fyn protein expression and quantitative analysis of its protein levels in with N-DPSCs and 50 ng/mL TNF- α stimulated DPSCs (TNF- α -DPSCs) was shown. **e** Fyn mRNA expression in N-DPSCs and TNF- α -DPSCs was detected by qRT-PCR. **f** Mineralization of odontogenic induction of N-DPSCs and TNF- α -DPSCs was observed by alizarin red and alkaline phosphatase (ALP) staining. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

we found that bone differentiation ability of DPSCs decreased was reduced under the stimulation of 50 ng/mL TNF- α , further verifying our stimulating concentration (Fig. 2f).

Fyn is a downstream target molecular of miR-125a-3p

To further investigate the upstream factors of Fyn, we used TargetScan, miRbase, microRNA.org and starBase V2.0 to perform computer analysis to predict miRNAs

that may target Fyn. Among the projected candidates, we focused on miR-125a-3p (Fig. 3a). Through extensive literature review, we found that such a mutually targeted relationship has been proved in many literatures (Ninio-Many et al. 2013; Tu et al. 2016; Xu et al. 2019). In order to verify its effect on caries, we detected miR-125a-3p in N-DPSCs,

I-DPSCs and TNF- α -DPSCs by qRT-PCR. It was found that the expression of miR-125a-3p in DPSC decreased significantly under the stimulation of inflammation (Fig. 3b).

In order to verify whether miR-125a-3p acts on Fyn, we overexpressed or knocked down miR-125a-3p in DPSC (Fig. 3c). At the same time, we found that

Fig. 3 Fyn is a downstream target molecular of miR-125a-3p. **a** Bioinformatics analysis was used to predict the target of miR-125a-3p and Fyn at www.mircroRNA.ORG.com. **b** qRT-PCR was used to detect miR-125a-3p content in N-DPSCs, I-DPSCs and TNF- α -DPSCs. DPSCs were over-expressed and inhibited by miR-125a-3p mimic and inhibitor respectively (mimic, inhibitor group), and treated with the same amount of PBS as a control group. **c** Transfection efficiency was detected by qRT-

PCR. **d** Correlation analysis of miR-125a-3p and Fyn. **e** Differential expression of Fyn protein and its protein level in the control, mimic, inhibitor group were detected by WB. **f** Immunofluorescence observation of the distribution of Fyn protein in siRNAs treated DPSCs (magnification $\times 200$). **g** Fyn mRNAs in the control, mimic, inhibitor group were analyzed by qRT-PCR. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

after overexpression of miR-125a-3p, the expression of Fyn decreased; and the expression of Fyn was obviously increased after the miR-125a-3p knockout (Fig. 3d, f, g). Comprehensive data analysis showed that miR-125a-3p was negatively correlated with Fyn (Fig. 3e). In addition, it was observed from immunofluorescence that the expression of Fyn decreased significantly after miR-125a-3p overexpression (Fig. 3f).

miR-125a-3p as a positive factor regulating the odontoblastic differentiation of DPSCs

We examined the effect of miR-125a-3p and Fyn on odontoblastic by detecting NRP1, DSPP and DMP-1 expression after overexpressing or knocking down miR-125a-3p. In the previous literature, our team has verified the role of NRP1 in odontoblast differentiation and determined that Fyn is used as the upstream of NRP1 to regulate odontoblast differentiation through the classical Wnt/ β -catenin signaling (Song et al. 2017, 2018). In this study, we found that after overexpression of miR-125a-3p, Fyn expression decreased, while NRP1, DSPP and DMP-1 expression increased; after knocking down miR-125a-3p, Fyn expression increased, NRP1, DSPP and DMP-1 expression decreased (Figs. 3f, 4a, b). The result of qRT-PCR is the same (Fig. 4c–e). Alizarin red staining and ALP staining also further verified that the ability of DPSCs odontoblastic differentiation was increased after overexpression of miR-125a-3p and decreased after miR-125a-3p knockdown (Fig. 4f). These results indicate that miR-125a-3p can target Fyn and regulate NRP1, further regulating the odontoblastic differentiation of DPSCs. In addition, we detected the expression of IL-6 and IL-8 in cell supernatant after overexpressing or knocking down miR-125a-3p. We found that the expression of IL-6 and IL-8 decreased with the increase of the expression of miR-125a-3p; the expression of IL-6 and IL-8 increased with the decrease of the expression of miR-125a-3p (Fig. 4g).

Discussion

In this study, we first examined the upregulation of Fyn expression in the inflammatory environment of deep caries and succeeded in imitating the

inflammatory microenvironment by 50 ng/mL TNF- α . Subsequently, it was observed that miR-125a-3p expression was down-regulated in deep caries, and the negative correlation with Fyn was found by overexpressing and knocking down miR-125a-3p. Finally, we further examined the effect of miR-125a-3p in DPSCs odontoblastic differentiation.

As we know, the development of dental caries is due to the ability of dentin reparation less than destruction. Through previous studies, we found the role of Fyn-NRP1 interaction in the development of dental caries (Song et al. 2017, 2018). In order to further explore its regulatory mechanism, we speculate that miRNA can play an important role in the development of dental caries by regulating the expression of Fyn and selecting miR-125a-3p as the key research object. Previous studies have shown that miR-125a-3p may be a potential diagnostic marker for rheumatoid arthritis. Downregulation of miR-125a-3p expression involved in inflammation regulation (Kumar and Nerurkar 2014; Monk et al. 2010).

MiR-125 has long been widely recognized as a biomarker for many cancers (Hashiguchi et al. 2012; Jiang et al. 2010). Recent research results showed that miR-125 was related to cell stress, inflammatory reaction and pain, including ultraviolet radiation stress, rheumatoid arthritis, virus or bacteria-induced neuroinflammation (Kumar and Nerurkar 2014; Murata et al. 2013; Tan et al. 2012). Detection of miR-125a in plasma may be a new diagnostic basis for rheumatoid arthritis (Murata et al. 2013). The expression of miR-125a-3p was down-regulated in studies of neuroinflammation in mice (Kumar and Nerurkar 2014). In the study of rat orofacial inflammatory pain, it was found that miR-125a-3p is negatively correlated with the development and maintenance of orofacial inflammatory pain via regulating p38/MAPK (Dong et al. 2014).

Fyn, the Src-family kinase (SFK), is expressed in a broad range of tissues and is downstream of important cell surface receptors. Fyn has been shown to mediate proinflammatory mediator production in a variety of cells, which acts as a major upstream regulator of proinflammatory signaling involving the MAPK and NF- κ B pathways (Panicker et al. 2015; Rajasekaran et al. 2013). According to the prediction website, we found that miR-125a-3p was closely related to the regulation of Fyn. In the study of tumors, it was confirmed that miR-125a-3p negatively regulates Fyn

Fig. 4 miR-125a-3p as a positive factor regulating the odontoblastic differentiation of DPSCs. N-DPSCs were treated with miR-125a-3p control, mimic and inhibitor and then cultured in odontogenic differentiation medium for 21 days. **a** Western blot analysis of the expression of odontoblast differentiation-related proteins DSPP, DMP1 and the expression of Fyn downstream target protein NRP1 in the control, mimic, inhibitor group.

b Quantitative analysis of protein levels mRNA levels of DSPP, DMP-1, and NRP1. **c-e** The mRNA expression of DSPP, DMP-1 and NRP1 was detected by qRT-PCR. **f** The effect of miR-125a-3p overexpression and knockdown on mineralization capacity of DPSC was observed by alizarin red and ALP staining. **g** The contents of IL-6 and IL-8 in cell supernatant were detected by ELISA. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Fig. 5 Schematic showing the signaling pathway of miR-125a-3p-Fyn-NRP1 interaction by TNF- α in DPSCs. As the rickets progress, the inflammatory stimuli increase, the expression of miR-125a-3p decreases, the expression of Fyn increases, and the interaction between NRP1 and FYN is promoted, resulting in the

original differentiation-promoting effect being masked. Instead, NRP1-FYN is formed. Complex, interaction promotes downstream NF- κ B signaling activation, further enlarges inflammatory response, inhibits dentin differentiation of DPSCs

involved in various physiological and pathological processes (Ninio-Many et al. 2014; Ninio-Many et al. 2013).

In conclusion, miR-125a-3p may be negatively correlated with the severity of dental caries. In caries, pathogens invaded the medullary cavity can cause the expression of miR-125a-3p increased in DPSCs by the release of various inflammatory factors, resulting in Fyn upregulation. Fyn interacts with NRP1 to form a NRP1-Fyn compound and then activates the NF- κ B signaling pathway. As a result, the odontoblastic differentiation ability of DPSCs is decreased, and the cascade of further expanding inflammatory effects appear, eventually destroying normal tooth tissues and aggravating the progression of dental caries (Fig. 5).

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Compliance with ethical standards

Conflict of interest The authors declare that no conflicting financial interests exist.

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