

RESEARCH

Open Access



A potential pathogenic hypoxia-related gene HK2 in necrotizing enterocolitis (NEC) of newborns

Yujie Han^{1†}, Xianghong Liu^{1†}, Lili Kang¹, Dong Chen¹, Yongqing Li², Huiping Zhang¹, Mingying Sun¹, Hui Gao¹, Zhongtao Gai^{1*} and Xiaoying Li^{1*}

Abstract

Background: Necrotizing enterocolitis (NEC) is a disastrous gastrointestinal disease of newborns, and the mortality rate of infants with NEC is approximately 20%-30%. The exploration of pathogenic targets of NEC will be conducive to timely diagnosis of NEC.

Methods: The whole transcriptome RNA sequencing was performed on NEC samples to reveal the expression of lncRNAs, circRNAs, miRNAs and mRNAs. Using differential expression analysis, cross analysis, target prediction, enrichment analysis, the pathogenic ceRNA network and target was found.

Results: Preliminarily, 281 DEmRNAs, 21 DEmiRNAs, 253 DElncRNAs and 207 DEcircRNAs were identified in NEC samples compared with controls. After target prediction and cross analyses, a key ceRNA regulatory network was built including 2 lncRNAs, 4 circRNAs, 2 miRNAs and 20 mRNAs. These 20 mRNAs were significantly enriched in many carbohydrate metabolism related pathways. After cross analysis of hypoxia-, carbohydrate metabolism-related genes, and 20 core genes, one gene HK2 was finally obtained. Dendritic cells activated were significantly differentially infiltrated and negatively correlated with HK2 expression in NEC samples.

Conclusions: The promising pathogenic hypoxia-related gene HK2 has been firstly identified in NEC, which might also involve in the carbohydrate metabolism in NEC.

Keywords: Necrotizing enterocolitis, HK2, Hypoxia, Carbohydrate metabolism, Whole transcriptome RNA sequencing

Background

Necrotizing enterocolitis (NEC) is a disastrous gastrointestinal disease of newborns, especially affecting those neonates with very-low birth weight (< 1500 g) [1]. Although the management of preterm infants has been much improved, the incidence of NEC among

newborns is still worrying [2]. The beginning symptoms of NEC might be slow and insidious, such as feeding intolerance, while they can rapidly develop into a fulminant NEC [3]. The mortality rate of infants with NEC is approximately 20%-30%, and the rest survivors will face many short- or long-term complications, including poor growth, intestinal problems, and so on [4, 5]. Multiple risk factors involve in the progression of NEC, comprising prematurity, intestinal bacterial dysbiosis, formula feeds, etc. [6]. Moreover, systemic hypoxia and intestinal ischemia are main stresses leading to NEC [7]. However, potential pathogenesis of NEC complicatedly results from many factors, such as the imbalance

[†]Yujie Han and Xianghong Liu contributed equally to this work.

*Correspondence: gztjn@outlook.com; lixiaoyingsd2020@163.com

¹ Department of Neonatal, Children's Hospital Affiliated to Shandong University/Jinan Children's Hospital, No. 23976 Huaiyin District, Jinan, 250022 Shandong, People's Republic of China

Full list of author information is available at the end of the article



between anti-inflammatory and proinflammatory factors [8], which still remains to be clarified. Additionally, due to nonspecific symptoms of NEC, it is hard to make early clinical diagnosis and distinguish NEC from other diseases with similar features [9]. Therefore, the exploration of promising pathogenic targets of NEC will be conducive to the timely diagnosis of NEC, indirectly contributing to better prognosis of NEC newborns.

Prematurity and enteral feeding are known as two main risk factors for NEC [10]. The primary transport function of intestinal epithelial cells is driven by consuming great amounts of energy and oxygen [11]. The digestion and nutrient transport after feeding demands more energy and oxygen. Intestinal epithelium is suffering from dynamic splanchnic circulation and anoxic environment of intestinal lumen at the same time [12], resulting in a highly fluctuating oxygen supply, thereby leading to intestinal mucosa hypoxia [11]. It has been evidenced that the synergistical role of feeding and postprandial hypoxia leads to the intestinal hypoxia in NEC [7]. Moreover, formula feeding has been reported to significantly associate with the intestinal hypoxia in NEC [13]. Compared with spontaneous intestinal perforation samples, the hypoxia related genes are significantly upregulated in human NEC samples [14]. Additionally, hypoxia and gavage feeding treatments are commonly used for NEC mouse model construction [15]. More importantly, hypoxia inducible factors (HIFs), HIF-1 and HIF-2, play crucial roles in many inflammatory bowel diseases [16]. However, the interaction of coding RNAs and non-coding RNAs has been seldom reported in hypoxia of NEC.

With the great development of next generation sequencing, whole transcriptome RNA sequencing has been contributing to reveal the crucial role of coding RNAs and non-coding RNAs in various diseases, including NEC. Herein, the purpose of our study is to explore the important pathogenic genes involving hypoxia in NEC via combining whole transcriptome RNA sequencing and further bioinformatics analyses.

Methods

Specimen collection

A total of 6 clinical samples were collected from Children's Hospital Affiliated to Shandong University/Jinan Children's Hospital, including 3 NEC tissue samples and 3 normal tissue samples. Our experiments were approved by ethic committee of Children's Hospital Affiliated to Shandong University/Jinan Children's Hospital, in accordance with The Helsinki Declaration. The written informed consents were obtained from the guardians of all subjects.

Data resources

GSE46619 dataset was downloaded from Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). Among all samples in this dataset, NEC tissue samples and normal intestinal tissue samples were picked as the validation dataset, including 5 NEC samples and 4 normal samples.

Moreover, hypoxia gene set (200 genes) and carbohydrate metabolism gene set (286 genes) were obtained from the MSigDB database (<https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>).

RNA extraction and sequencing

Total RNA was extracted from the tissue samples with miRNeasy Tissue/Cells Advanced Micro Kit (Qiagen, NO.217684, Shenzhen, China), and detected on Nanodrop2000. The RNA integrity was assessed by agarose gel electrophoresis, and RIN was detected on Agilent2100. The mRNA sequencing was conducted on MGISEQ2000 platform, and the reagents used included Dynabeads[®] mRNA Purification Kit (for mRNA Purification from Total RNA Preps, Invitrogen, NO.61006, Shanghai, China), Library Preparation VAHTS mRNA Capture Beads (Vazyme, N401-01-02, Nanjing, China), and MGIEasy Duplex UMI Universal Library Prep Set (MGI, NO.1000006383, Shenzhen, China). Next, the raw data undergone quality control in SeqPrep software (<https://github.com/jstjohn/SeqPrep>) in order to obtain highly qualified sequences for subsequent analysis.

Differentially expressed RNA analysis

The differential expression analysis was performed in limma function [17] of R language (version 4.1.0, same below). The significantly differentially expressed mRNAs (DEmRNAs), miRNAs (DEmiRNAs), lncRNAs (DElncRNAs), and circRNAs (DEcircRNAs) between NEC and normal samples were screened with $|\log_2FC| > 2$ and P value < 0.05 .

Target relationship prediction

The target miRNAs of DEcircRNAs were predicted using CircNA database (<https://awi.cuhk.edu.cn/CircNAet/php/index.php>) to obtain circr-pre-miRNAs, and the structure of circRNAs, miRNA response element (MRE), and RNA-binding protein (RBP) information were obtained from Cancer-Specific CircRNA (CSCD) database (<http://gb.whu.edu.cn/CSCD2/#>). The miRcode database (<http://www.mircode.org>) was used for the targeted miRNAs prediction of DElncRNAs (lncpre-miRNAs). Finally, the target genes (pre-mRNAs) of circr-pre-miRNAs and lncpre-miRNAs were predicted

in miRWalk database (<http://mirwalk.umm.uni-heidelberg.de/>).

Functional enrichment analysis

The crucial genes were then subjected to the enrichment analysis in “clusterProfiler” [18] in order to obtain more functional information, including Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. The GO and KEGG terms with P adjust < 0.05 (adjusted with Benjamini and Hochberg (BH) method) were considered significantly enriched terms.

Construction of ceRNA network

Basing on the predicted interaction pairs and differentially expressed RNAs, we have constructed a circRNA-lncRNA-miRNA-mRNA ceRNA network. The complex post-transcriptional regulation in NEC could be further exhibited through the ceRNA network.

The impact of key gene on inflammatory responses in NEC

The expression of crucial pro-inflammatory cytokines interleukin-1 β (IL-1 β), interleukin-6 (IL-6), Tumour Necrosis Factor- α (TNF- α) was compared between NEC samples and normal samples.

Additionally, the relative immune cell infiltration in NEC samples was analyzed using CIBERSORT [19]. According to deconvolution algorithm, the composition of immune infiltrating cell was characterized in CIBERSORT basing on gene expression matrix and preset 547 barcode genes.

Results

Identification of differentially expressed circRNAs, lncRNAs, miRNAs, and mRNAs in NEC

Firstly, basing on our sequencing data, the differentially RNAs were identified between NEC and control samples. There were totally 281 DEmRNAs between NEC vs. control samples, comprising 103 upregulated DEmRNAs and 178 downregulated DEmRNAs (Fig. 1A), the expressions of which were significantly different (Fig. 1B). Considering the potential role of these 281 DEmRNAs in the onset of NEC, GO and KEGG functional enrichment was then conducted (The pathways were obtained basing on KEGG [20–22]). The 281 DEmRNAs were significantly enriched in 207 GO terms (the top 10 terms were displayed in Fig. 1C), and 6 KEGG pathways (Fig. 1D), such as IL-17 signaling pathway. The detailed functional results were listed in Table S1.

Next, differentially expressed circRNAs, lncRNAs, miRNAs were also found between NEC vs. control samples. A total of 21 DEmiRNAs, 253 DELncRNAs and 207 DECircRNAs were found between NEC vs. control samples. Compared with control samples, 14 upregulated

and 7 downregulated DEmiRNAs (Fig. 2A), 61 upregulated and 192 downregulated DELncRNAs (Fig. 2B), and 59 upregulated and 148 downregulated DECircRNAs (Fig. 2C) were identified in NEC specimens. The expression levels of DEmiRNAs, DELncRNAs, and DECircRNAs were significantly different between NEC vs. control samples, separately (Fig. 2D–F).

Targeted miRNAs prediction of DELncRNAs and DECircRNAs

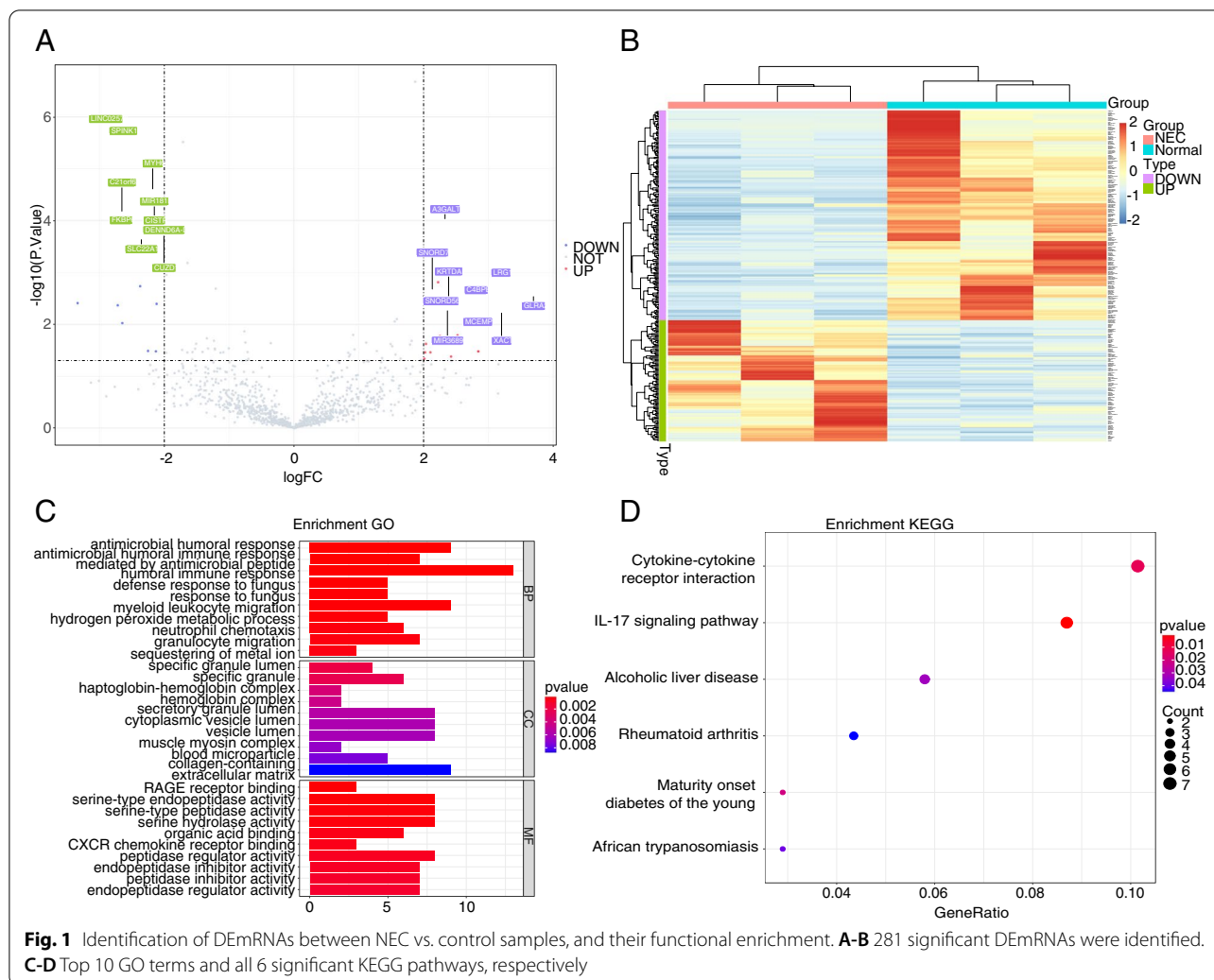
The targeted miRNAs of 253 DELncRNAs were predicted with miRcode database. Then regulatory pairs between 17 lncRNAs and 142 miRNAs were obtained. After cross analysis of 142 miRNAs and 21 DEmiRNAs, 5 overlapped miRNAs (lnc-pre-miRNAs) were found (Fig. 3A), including hsa-miR-124-5p, hsa-miR-222-5p, hsa-miR-518e-5p, hsa-miR-520 g-5p, hsa-miR-519-5p.

The targeted miRNAs of 207 DECircRNAs were analyzed using circNet database and chromosomal localization. The regulatory pairs between 40 circRNAs and 250 miRNAs were identified. There were 3 overlapped miRNAs (circ-pre-miRNAs) between 250 miRNAs and 21 DEmiRNAs, including hsa-miR-222-5p, hsa-miR-522-5p, hsa-miR-520 g-5p (Fig. 3B). Basing on these 3 overlapped miRNAs, the host gene information of the corresponding 4 circRNAs was obtained, which were located on chromosomes 5, 7, 16 and 17. Moreover, the structural information and CircBaseID of these 4 circRNAs were analyzed using CSCD database, including hsa_circ_0001522 (Fig. 3C), hsa_circ_0000690 (Fig. 3D), hsa_circ_0001772 (Fig. 3E) and hsa_circ_0004273 (Fig. 3F).

Construction of key ceRNA regulatory network

Subsequently, the target genes (pre-mRNAs) of 5 lnc-pre-miRNAs and 3 circ-pre-miRNAs were identified using miRwalk database, comprising 5989 and 4944 pre-mRNAs, respectively. To further find those genes related the onset of NEC, pre-mRNAs and 281 DEmRNAs were then subjected to a cross analysis, and 24 crucial genes were identified (Fig. 4A).

Basing on these 24 crucial overlapped genes, a 4 lncRNAs-4 miRNAs-24 mRNAs' network (Fig. 4B) and a 4 circRNAs-3 miRNAs-24 mRNAs' network (Fig. 4C) were constructed. Among them, there were 2 overlapped miRNAs and corresponding 20 mRNAs. Thus, a key ceRNA regulatory network was built including 2 lncRNAs (LINC00402 and CYB561D2), 4 circRNAs (hsa_circ_0001522, hsa_circ_0000690, hsa_circ_0001772, and hsa_circ_0004273), 2 miRNAs (miR-222-5p and miR-520 g-5p) and 20 mRNAs (Fig. 4D), which played a crucial role in the onset of NEC.



Functional enrichment analysis of core genes in NEC

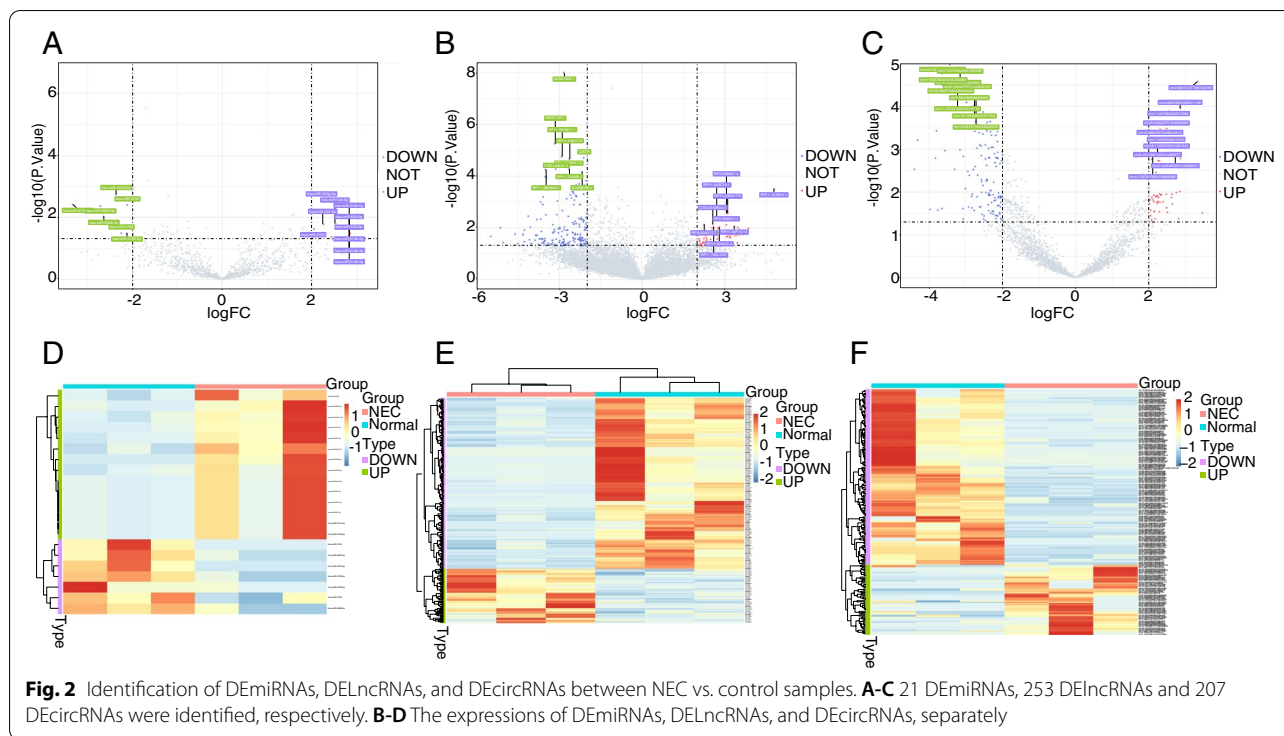
Due to the potentially important role of the ceRNA network in NEC, the 20 mRNAs then undergone GO and KEGG functional enrichment analysis. We found that 38 GO terms, involving Regulation of glucose transmembrane transport, Myeloid leukocyte migration, etc., were significantly (top 21 terms showed in Fig. 5A). These 20 mRNAs were significantly enriched in 13 KEGG pathways, such as Galactose metabolism, Starch and sucrose metabolism, Carbohydrate digestion and absorption, and so on (Fig. 5B). All enrichment results were shown in Table S2.

Crucial gene HK2 involving carbohydrate metabolism and hypoxia in NEC

The functional enrichment results of the 20 core genes in ceRNA network implied many carbohydrate metabolism related pathways were significantly enriched, including

Galactose metabolism, Fructose and mannose metabolism, Starch and sucrose metabolism, Biosynthesis of nucleotide sugars, Carbohydrate digestion and absorption, and Amino sugar and nucleotide sugar metabolism. Moreover, undigested carbohydrate fermentation in bowel has been demonstrated to yield short chain organic acids, thereby leading to intestinal mucosa disruption and inflammation [23]. The functional information inspired us regarding the core genes' impacts on carbohydrate metabolism in NEC. On the other hand, combining the crucial role of hypoxia in NEC [13], the crucial genes involving carbohydrate metabolism and hypoxia in NEC were screened, and the whole screening process was shown in Fig. 6.

Then hypoxia related gene set (200 genes) and carbohydrate metabolism gene set (286 genes) were downloaded from MSigDB database (Table S3). After cross analysis of hypoxia related gene set, carbohydrate metabolism gene



set, and 20 core genes, one gene HK2 was finally obtained (Fig. 7A).

In our sequencing data, HK2 was significantly highly expressed in NEC tissues comparing with controls (Fig. 7B). The expression of HK2 was also evaluated in an independent cohort GSE46619, and significantly higher HK2 expression was also observed in NEC samples ($p < 0.01$) (Fig. 7C). Collectively, HK2 was a probably crucial pathogenic gene in NEC involving carbohydrate metabolism and hypoxia.

Impact of HK2 on inflammatory responses in NEC

The enhancement of immune and inflammation responses are important hallmarks of NEC [24]. Thus, the potential impacts of HK2 on inflammatory responses in NEC were also analyzed herein. Firstly, crucial pro-inflammatory cytokines’ expression was compared between NEC vs. Controls using our sequencing data. Our results indicated that the expression levels of IL-1 β and IL11 showed higher expressions in NEC samples compared with normal samples (Fig. 8A-B). Moreover, the same tendency was also found in GSE46619 dataset (Fig. 8C-D). Our data suggested that inflammatory responses were probably activated in NEC samples.

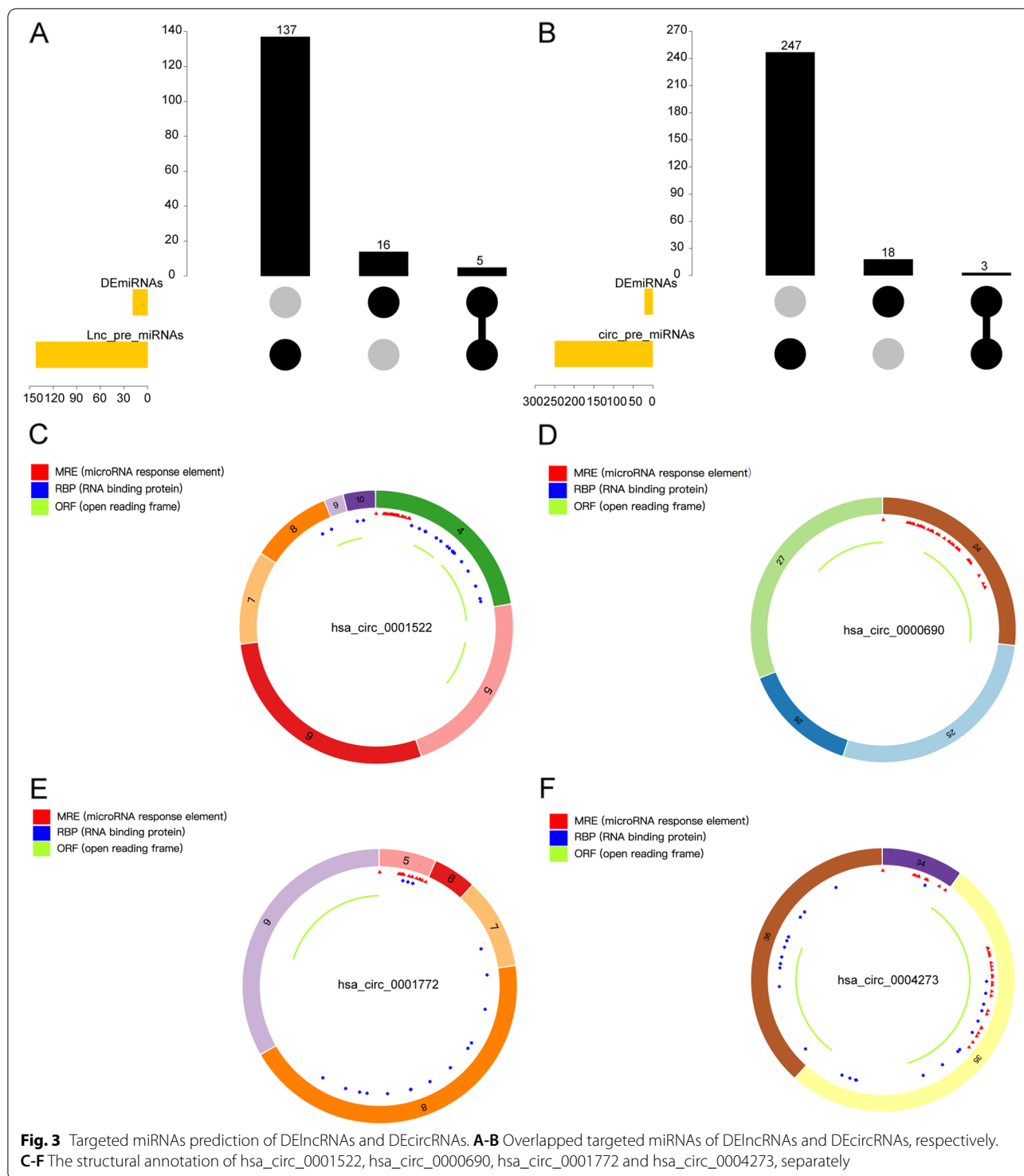
Additionally, basing on our sequencing data, the immune cell infiltration in all samples was also evaluated in CIBERSORT to get more information regarding immune responses. The general results of 22 types of

immune cells’ infiltration in NEC and control samples were shown in Fig. 8E, indicating the individual characteristics. Additionally, we found that two types of immune cells, NK cells resting and Dendritic cells activated were significantly differentially infiltrated between NEC vs. Controls (Fig. 8F-G), which probably contributed to the development of NEC. Besides, in NEC specimens, there was significant negative correlation between HK2 and infiltration of Dendritic cells activated (Fig. 8H).

Discussion

As a leading cause of neonatal mortality, NEC has been a great healthy threat to neonates, especially to premature infants [25]. However, to date, the comprehensive analysis of mRNAs, miRNAs, lncRNAs, and circRNAs in NEC tissue specimens of newborns is quite limited. Accordingly, we herein utilized the whole transcriptome RNA sequencing and further bioinformatics mining to study the potential pathogenic ceRNA network and crucial gene in NEC. HK2 was found to involve in hypoxia and carbohydrate metabolism in NEC.

Although the role of some non-coding RNAs has been explored in NEC rat models, for example, miR-27a-5p and miR-187-3p are probably involving the NEC pathophysiological mediation through Wnt signaling [6]. However, little is known about the role of lncRNAs, circRNAs, and their interaction with mRNAs. Accordingly,



whole transcriptome RNA sequencing was conducted to revealed the potential pathogenic non-coding RNAs and mRNAs in NEC. Preliminarily, 281 DEmiRNAs, 21 DEmiRNAs, 253 DELncRNAs and 207 DEcircRNAs

were identified in NEC samples. The enrichment analysis indicated that the 281 DEmiRNAs were significantly enriched in IL-17 signaling pathway. IL-17 signaling pathway is able to trigger several pro-inflammatory

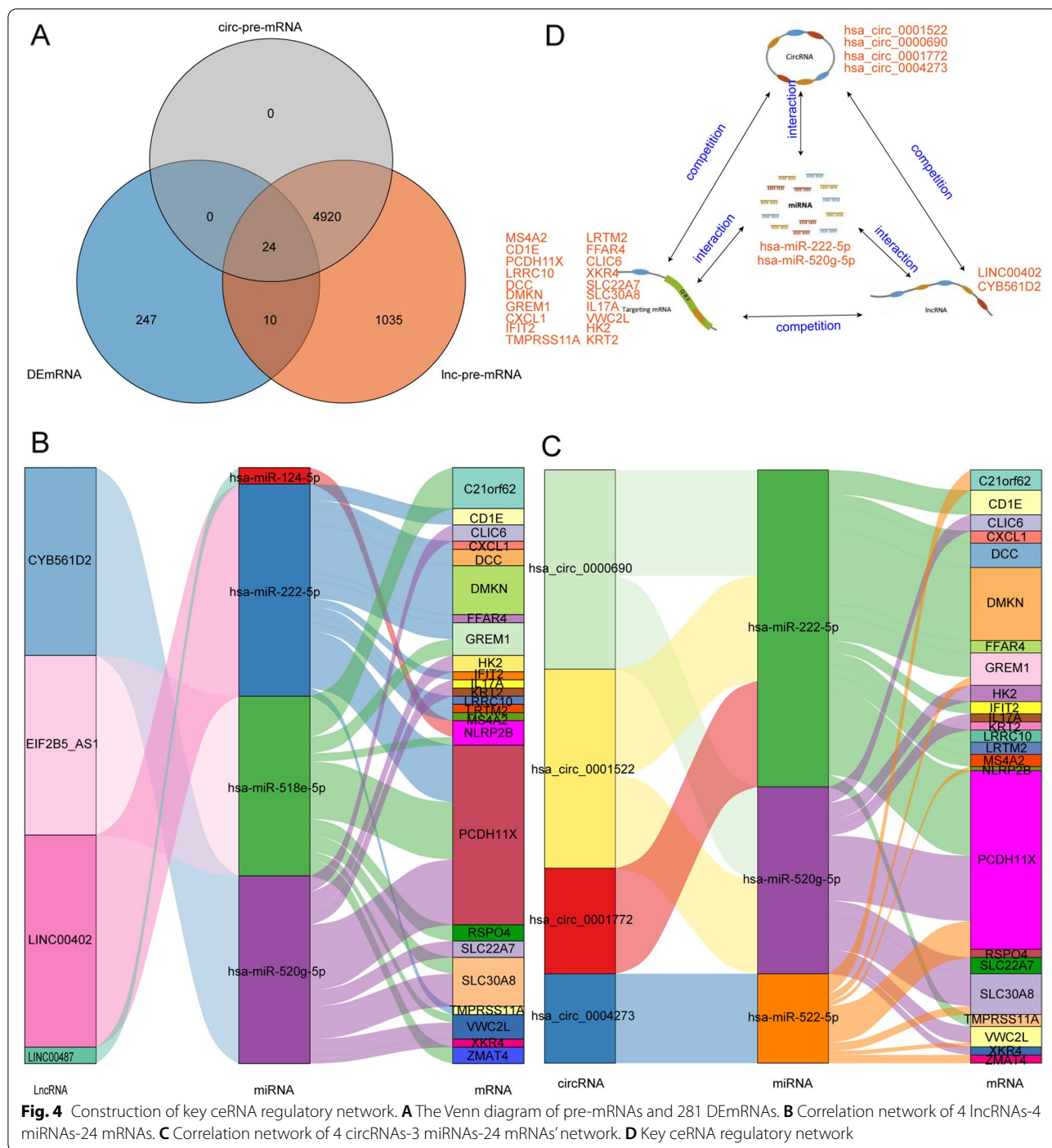


Fig. 4 Construction of key ceRNA regulatory network. **A** The Venn diagram of pre-mRNAs and 281 DEmRNAs. **B** Correlation network of 4 lncRNAs-4 miRNAs-24 mRNAs. **C** Correlation network of 4 circRNAs-3 miRNAs-24 mRNAs' network. **D** Key ceRNA regulatory network

molecule events, such as IL22, IL1 β , TNE, and so on [26]. The main pathophysiology of NEC has been known as extreme inflammatory responses and necrosis [27]. Although IL-17 signaling has been illustrated in some IBDs like Crohn's disease [26], it has been seldom studied in NEC. Our data provided more evidence regarding

inflammatory responses in NEC. Furthermore, we found that proinflammatory cytokines, IL-1 β and IL11, showed higher expression in NEC. Besides, Dendritic cells (DCs) activated were significantly differentially infiltrated between NEC vs. Controls, and negatively correlated with the HK2 expression in NEC samples.

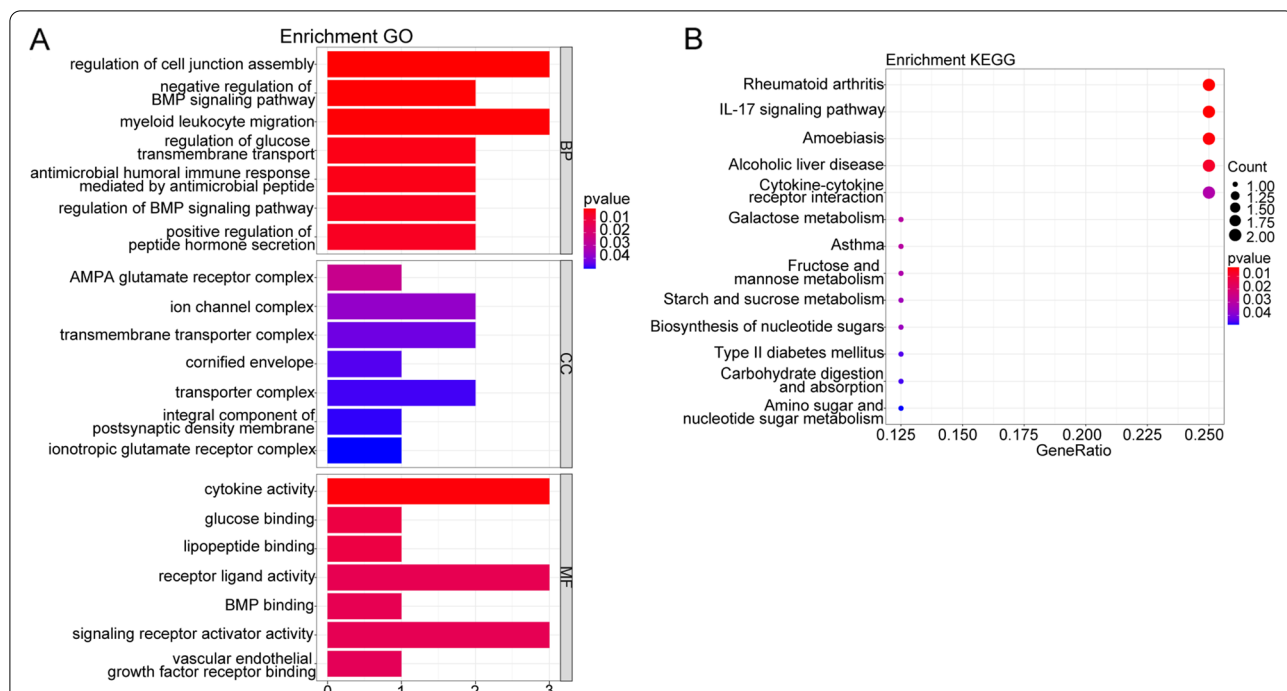


Fig. 5 GO and KEGG functional enrichment analysis of 20 core mRNAs. **A** The top 21 significantly enriched GO terms. **B** 13 significantly enriched KEGG pathways

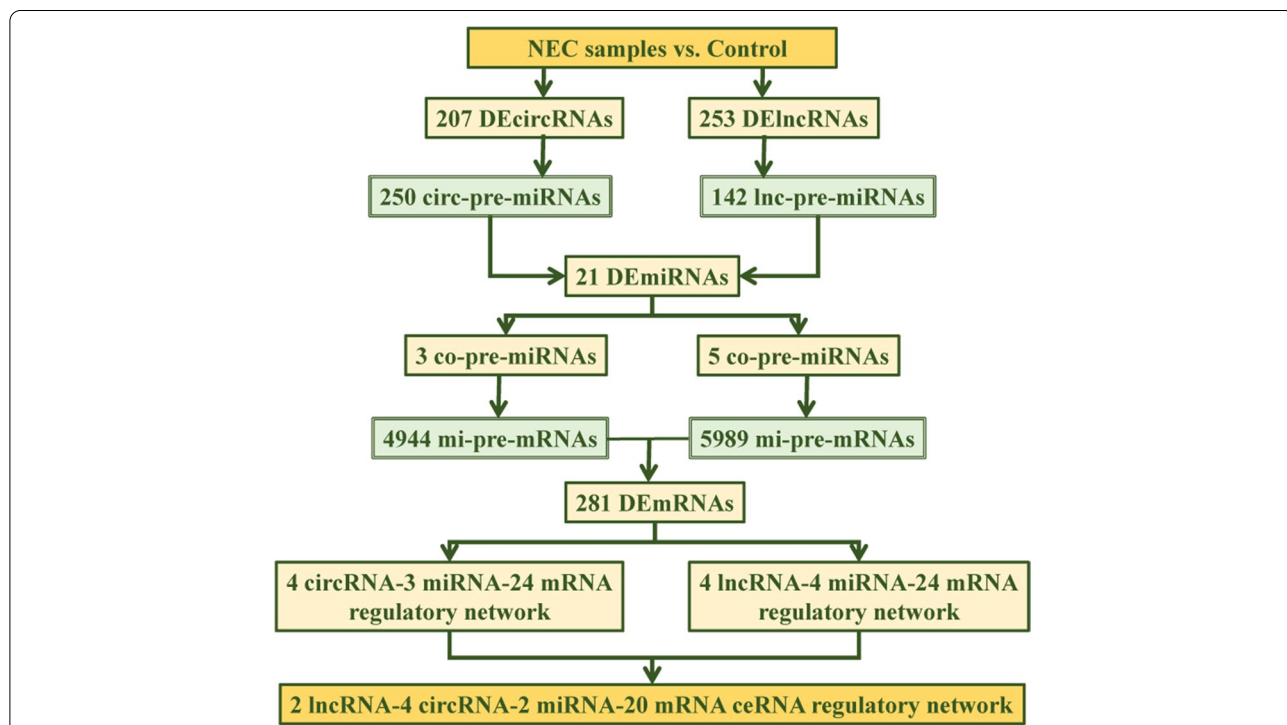
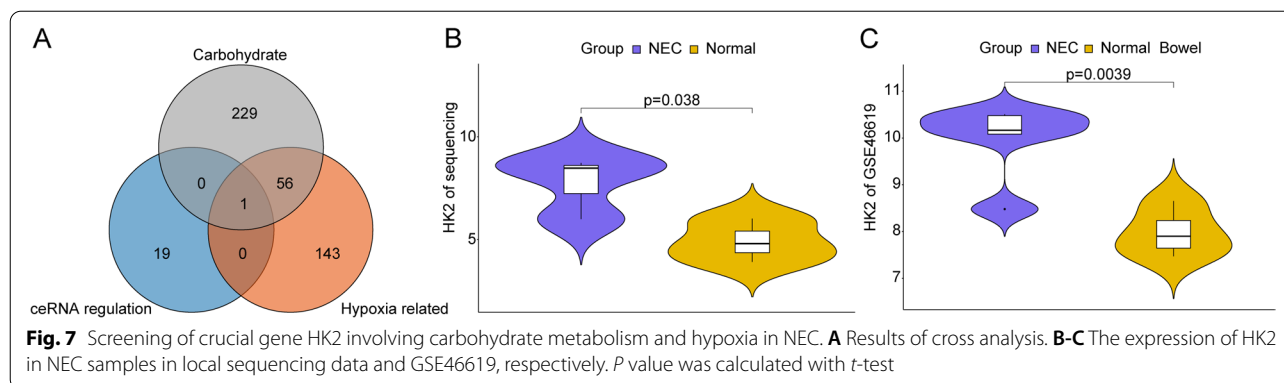


Fig. 6 Key gene screening process



Emami et al. have reported that in NEC mouse models, *C. sakazakii* infection could destruct the intestinal epithelium via recruiting more DCs and suppressing the DC maturation [28]. Obviously, lower levels of activated DCs seemed to exert unfavorable effects on NEC.

Subsequently, after target prediction and cross analyses basing on the differentially expressed RNAs, a key ceRNA regulatory network was built including 2 lncRNAs (LINC00402 and CYB561D2), 4 circRNAs, 2 miRNAs (miR-222-5p and miR-520 g-5p) and 20 mRNAs. Most of the non-coding RNAs are reported in NEC for the first time in our study. Further functional information of these 20 key genes was obtained, involving many carbohydrate metabolism related pathways. Additionally, considering the important effects of hypoxia on NEC of newborns, the core gene HK2 was finally screened involving carbohydrate metabolism and hypoxia in NEC. Although limited reports directly supported the interaction between HK2 and these non-coding RNAs, especially in NEC, some indirect clues could be found. LncRNA CYB561D2 has been indicated to activate STAT3 and lead to the immunosuppression in brain tumor [29]. Meanwhile, hypoxia was a crucial factor driving immunosuppression in various tumors [30]. However, whether CYB561D2 and HK2 could exert similar roles under hypoxia in NEC should be further investigated. Recently, in a breast cancer cell line, miR-222-5p has been evidenced to be downregulated under hypoxia [31], indicating that miR-222-5p expression indeed was affected by hypoxia. Whereas, the interaction between HK2 and miR-222-5p under hypoxia still needs to be further clarified in NEC in the future.

Hexokinases (HKs) catalyze glucose to yield glucose-6-phosphate (G6P) involving the first committed step in glucose metabolism, and HK2 (hexokinase 2) is a member of hexokinase family [32]. Although HK2 has been seldom studied in NEC of newborns, the role of HK2 has been illustrated in many diseases. Han et al. have recently demonstrated that differential DNA methylation/ hydroxymethylation and gene expression of HK2 was observed in mouse model of inflammatory bowel disease (IBD) [33]. Whereas, as another intestinal inflammatory disease, whether similar epigenetic modification of HK2 occurs in NEC still needs to be clarified. Additionally, in hepatomas, HK2 has been found to be highly expressed in tumor cells, accompanied with great G6P production, and G6P was an important carbon and energy source in hypoxic conditions [34]. More importantly, as a crucial transcription factor in hypoxia, HIF-1 α is able to bind with the promoter of HK2 to promote the transcription of HK2 [35]. Thus, we suspect that in NEC, HK2 exerts adaptive role in providing carbon and energy source in intestinal epithelium in a dynamic oxygen conditions, involving hypoxia and carbohydrate metabolism. Additionally, Pavo et al. have recently reported their findings in repetitive ischemia/reperfusion model that HK2 was significantly upregulated in ischemic zone while downregulated in heart regions, indicating the intrinsic remote ischemic conditioning (RIC) of myocardium [36]. In NEC, RIC has been increasingly considered a promising tool to protect distant organs from ischemia/ hypoxia-induced damage [37]. Accordingly, their work inspired us to

(See figure on next page.)

Fig. 8 Potential impacts of HK2 on inflammatory responses in NEC. **A-B** In our sequencing data, the expression levels of IL-1 β and IL11, respectively. **C-D** In dataset GSE46619, the expression levels of IL-1 β and IL11, respectively. **E** The immune cell infiltration in our local samples. **F-G** Dendritic cells activated and NK cells resting were significantly differentially infiltrated between NEC vs. controls, separately. **H** The correlation analysis of HK2 and Dendritic cells activated. *P* value was calculated with *t*-test

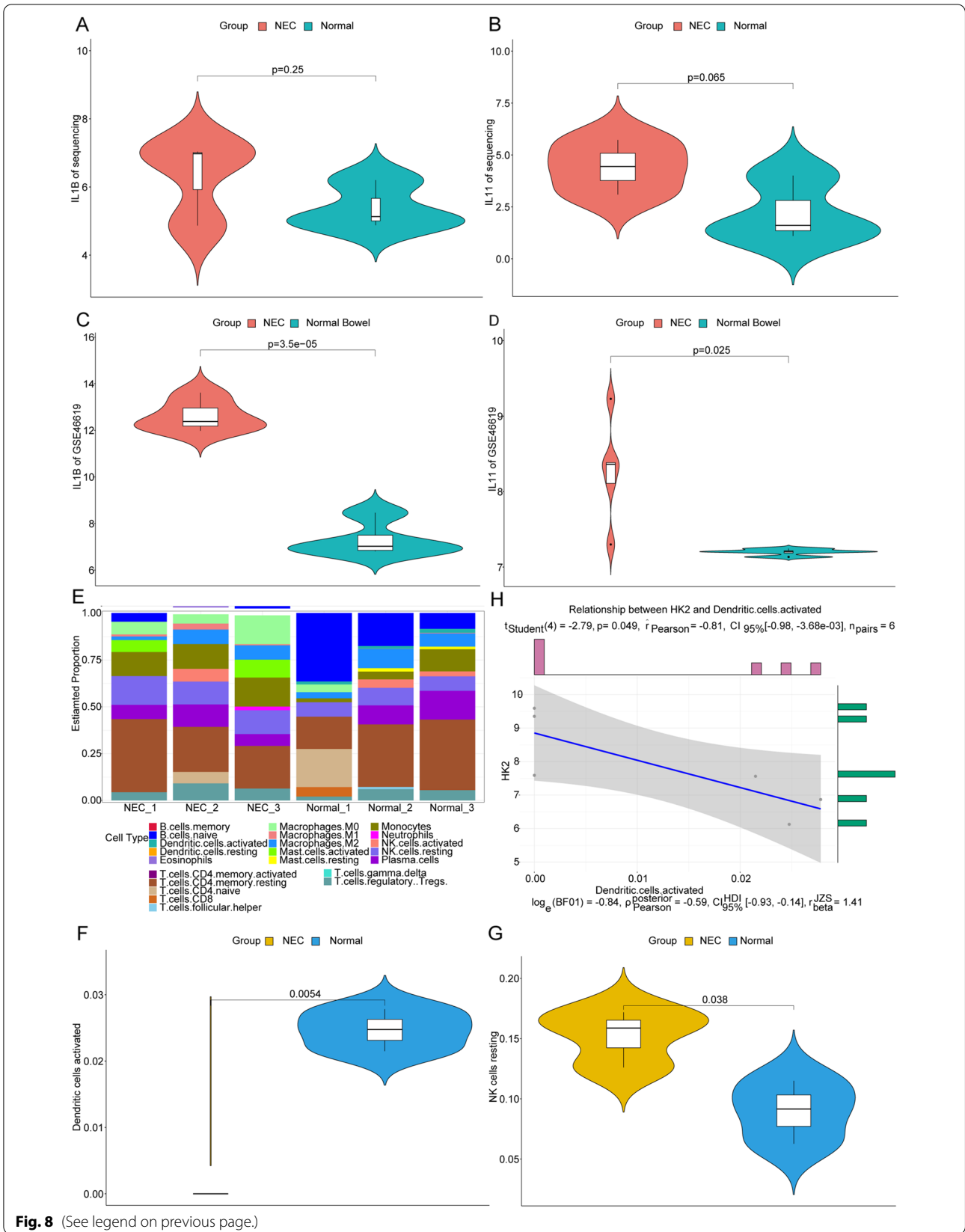


Fig. 8 (See legend on previous page.)

connect HK2 with RIC, which deserved subsequent investigation in the near future.

Finally, it is still urgently needed to reveal the HK2 related underlying mechanisms in NEC, in order to better understand the detailed pathogenic role of HK2. Only then it will be possible to further explore its role in larger sample size and other sample type, such as blood sample. All above work are expected to help us to apply our findings to clinical cases earlier. The hypoxia-related gene HK2 we identified is promising to provide more alternatives for early detection and diagnosis of NEC.

Conclusions

In summary, basing on the whole transcriptome RNA sequencing analysis of our local clinical samples, we fully use bioinformatics tools and mine the potential pathogenic ceRNA network in NEC. Notably, a promising pathogenic hypoxia-related gene HK2 has been firstly identified in NEC, involving the carbohydrate metabolism. Our findings give more insights into understanding the pathogenesis of NEC of newborns.

Abbreviations

NEC: Necrotizing enterocolitis; HIFs: Hypoxia inducible factors; GEO: Gene Expression Omnibus; MRE: miRNA response element; RBP: RNA-binding protein; CSCD: Cancer-Specific CircRNA; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; IL-1 β : Interleukin-1 β ; IL-6: Interleukin-6; TNF- α : Tumour Necrosis Factor- α .

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12887-022-03664-w>.

Additional file 1. Detailed functional results of DE mRNAs.

Additional file 2. Detailed GO and KEGG functional results of core genes in NEC.

Additional file 3. All genes in hypoxia gene set and carbohydrate metabolism gene set.

Acknowledgements

Not applicable.

Authors' contributions

Yujie Han and Xianghong Liu contributed to the conception of the study and wrote the manuscript. Yujie Han, Xianghong Liu, Zhongtao Gai and Xiaoying Li provided the study materials and clinical samples. Lili Kang, Dong Chen and Yongqing Li performed the data collection and analysis. Huiping Zhang, Mingying Sun and Hui Gao helped perform the experiment. All authors read and approved the final manuscript.

Funding

None.

Availability of data and materials

The datasets generated and analysed during the current study are available from <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA835160>.

Declarations

Ethics approval and consent to participate

The trial was conducted in accordance with the Declaration of Helsinki. The study was approved by Ethics Committee of Children's Hospital Affiliated to Shandong University/Jinan Children's Hospital and informed consent was taken from all individual participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Neonatal, Children's Hospital Affiliated to Shandong University/Jinan Children's Hospital, No. 23976 Huaiyin District, Jinan, 250022 Shandong, People's Republic of China. ²Department of Neonatal, LaoLing Maternity and Child Health Care Hospital, 118 Anju Road, Laoling County, Dezhou, Shandong Province 253600, People's Republic of China.

Received: 9 May 2022 Accepted: 30 September 2022

Published online: 26 October 2022

References

- Meister AL, Doheny KK, Travagli RA. Necrotizing enterocolitis: It's not all in the gut. *Exp Biol Med* (Maywood). 2020;245(2):85–95.
- Alsaied A, Islam N, Thalib L. global incidence of necrotizing enterocolitis: a systematic review and Meta-analysis. *BMC Pediatr*. 2020;20(1):344.
- Tanner SM, Berryhill TF, Ellenburg JL, Jilling T, Cleveland DS, Lorenz RG, Martin CA. Pathogenesis of necrotizing enterocolitis: modeling the innate immune response. *Am J Pathol*. 2015;185(1):4–16.
- Jones IH, Hall NJ. Contemporary outcomes for infants with necrotizing enterocolitis—a systematic review. *J Pediatr*. 2020;220(86–92):e83.
- Olaloye OO, Liu P, Toothaker JM, McCourt BT, McCourt CC, Xiao J, Prochaska E, Shaffer S, Werner L, Faculty UN, et al. CD16+CD163+ monocytes traffic to sites of inflammation during necrotizing enterocolitis in premature infants. *J Exp Med*. 2021;218(9):e20200344.
- Yu RQ, Wang M, Jiang SY, Zhang YH, Zhou XY, Zhou Q. Small RNA Sequencing Reveals Differentially Expressed miRNAs in Necrotizing Enterocolitis in Rats. *Biomed Res Int*. 2020;2020:5150869.
- Chen Y, Koike Y, Miyake H, Li B, Lee C, Hock A, Zani A, Pierro A. Formula feeding and systemic hypoxia synergistically induce intestinal hypoxia in experimental necrotizing enterocolitis. *Pediatr Surg Int*. 2016;32(12):1115–9.
- Egan CE, Sodhi CP, Good M, Lin J, Jia H, Yamaguchi Y, Lu P, Ma C, Branca MF, Weyandt S, et al. Toll-like receptor 4-mediated lymphocyte influx induces neonatal necrotizing enterocolitis. *J Clin Invest*. 2016;126(2):495–508.
- Chaaban H, Shin M, Sirya E, Lim YP, Caplan M, Padbury JF. Inter-alpha inhibitor protein level in neonates predicts necrotizing enterocolitis. *J Pediatr*. 2010;157(5):757–61.
- Walsh V, Brown JVE, Copperthwaite BR, Oddie SJ, McGuire W. Early full enteral feeding for preterm or low birth weight infants. *Cochrane Database Syst Rev*. 2020;12:CD013542.
- Ward JB, Keely SJ, Keely SJ. Oxygen in the regulation of intestinal epithelial transport. *J Physiol*. 2014;592(12):2473–89.
- Taylor CT, Colgan SP. Hypoxia and gastrointestinal disease. *J Mol Med (Berl)*. 2007;85(12):1295–300.
- Chen Y, Koike Y, Chi L, Ahmed A, Miyake H, Li B, Lee C, Delgado-Olguin P, Pierro A. Formula feeding and immature gut microcirculation promote intestinal hypoxia, leading to necrotizing enterocolitis. *Dis Model Mech*. 2019;12(12):dmm040998.
- Chen Y, Chang KT, Lian DW, Lu H, Roy S, Lakshmi NK, Low Y, Krishnaswamy G, Pierro A, Ong CC. The role of ischemia in necrotizing enterocolitis. *J Pediatr Surg*. 2016;51(8):1255–61.
- Ma F, Hao H, Gao X, Cai Y, Zhou J, Liang P, Lv J, He Q, Shi C, Hu D, et al. Melatonin ameliorates necrotizing enterocolitis by preventing Th17/Treg

- imbalance through activation of the AMPK/SIRT1 pathway. *Theranostics*. 2020;10(17):7730–46.
16. Kerber EL, Padberg C, Koll N, Schuetzhold V, Fandrey J, Winning S. The importance of Hypoxia-Inducible Factors (HIF-1 and HIF-2) for the pathophysiology of inflammatory bowel disease. *Int J Mol Sci*. 2020;21(22):8551.
 17. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*. 2015;43(7):e47.
 18. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284–7.
 19. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12(5):453–7.
 20. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28(1):27–30.
 21. Kanehisa M. Toward understanding the origin and evolution of cellular organisms. *Protein Sci*. 2019;28(11):1947–51.
 22. Kanehisa M, Furumichi M, Sato Y, Ishiguro-Watanabe M, Tanabe M. KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res*. 2021;49(D1):D545–51.
 23. Clark DA, Munshi UK. Feeding associated neonatal necrotizing enterocolitis (Primary NEC) is an inflammatory bowel disease. *Pathophysiology*. 2014;21(1):29–34.
 24. Zhang L, Sun L, Wu M, Huang J. Identification of Inflammatory Genes, Pathways, and Immune Cells in Necrotizing Enterocolitis of Preterm Infant by Bioinformatics Approaches. *Biomed Res Int*. 2021;2021:5568724.
 25. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, Bauer CR, Donovan EF, Korones SB, Laptook AR, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. *Am J Obstet Gynecol*. 2007;196(2):147 e141-148.
 26. Schmitt H, Neurath MF, Atreya R. Role of the IL23/IL17 Pathway in Crohn's Disease. *Front Immunol*. 2021;12:622934.
 27. Nino DF, Sodhi CP, Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. *Nat Rev Gastroenterol Hepatol*. 2016;13(10):590–600.
 28. Emami CN, Mittal R, Wang L, Ford HR, Prasadarao NV. Recruitment of dendritic cells is responsible for intestinal epithelial damage in the pathogenesis of necrotizing enterocolitis by *Cronobacter sakazakii*. *J Immunol*. 2011;186(12):7067–79.
 29. Tao B, Shi J, Shuai S, Zhou H, Zhang H, Li B, Wang X, Li G, He H, Zhong J. CYB561D2 up-regulation activates STAT3 to induce immunosuppression and aggression in gliomas. *J Transl Med*. 2021;19(1):338.
 30. Suthen S, Lim CJ, Nguyen PHD, Dutertre CA, Lai HLH, Wasser M, Chua C, Lim TKH, Leow WQ, Loh TJ, et al. Hypoxia-driven immunosuppression by Treg and type-2 conventional dendritic cells in HCC. *Hepatology*. 2022. PMID: 33923305.
 31. Camps C, Saini HK, Mole DR, Choudhry H, Reczko M, Guerra-Assuncao JA, Tian YM, Buffa FM, Harris AL, Hatzigeorgiou AG, et al. Integrated analysis of microRNA and mRNA expression and association with HIF binding reveals the complexity of microRNA expression regulation under hypoxia. *Mol Cancer*. 2014;13:28.
 32. Patra KC, Wang Q, Bhaskar PT, Miller L, Wang Z, Wheaton W, Chandel N, Laakso M, Muller WJ, Allen EL, et al. Hexokinase 2 is required for tumor initiation and maintenance and its systemic deletion is therapeutic in mouse models of cancer. *Cancer Cell*. 2013;24(2):213–28.
 33. Han Q, Kono TJ, Knutson CG, Parry NM, Seiler CL, Fox JG, Tannenbaum SR, Tretyakova NY. Multi-Omics Characterization of Inflammatory Bowel Disease-Induced Hyperplasia/Dysplasia in the Rag2(-/-)/Il10(-/-) Mouse Model. *Int J Mol Sci*. 2020;22(1):364.
 34. Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. *J Biol Chem*. 2001;276(46):43407–12.
 35. Cao L, Wang M, Dong Y, Xu B, Chen J, Ding Y, Qiu S, Li L, Karamfilova-Zaharieva E, Zhou X, et al. Circular RNA circRNF20 promotes breast cancer tumorigenesis and Warburg effect through miR-487a/HIF-1 α /HK2. *Cell Death Dis*. 2020;11(2):145.
 36. Pavo N, Lukovic D, Zlabinger K, Lorant D, Goliasch G, Winkler J, Pils D, Auer K, Ankersmit HJ, Giricz Z, et al. Intrinsic remote conditioning of the myocardium as a comprehensive cardiac response to ischemia and reperfusion. *Oncotarget*. 2017;8(40):67227–40.
 37. Koike Y, Li B, Ganji N, Zhu H, Miyake H, Chen Y, Lee C, Janssen Lok M, Zozaya C, Lau E, et al. Remote ischemic conditioning counteracts the intestinal damage of necrotizing enterocolitis by improving intestinal microcirculation. *Nat Commun*. 2020;11(1):4950.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

