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Dysregulation of MiR-199a/IL8 pathway in chronic Cr (VI)-induced tumor growth and angiogenesis

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ABSTRACT
Hexavalent chromium [Cr(VI)] is a well-known environmental carcinogen. Recent studies revealed that chronic exposure of human bronchial epithelial cells (BEAS-2B, B2B) to Cr(VI) activated several signaling pathways and induced cell malignant transformation and tumor growth. However, new mechanisms of Cr(VI) in inducing carcinogenesis remains to be elucidated. This study showed that miR-199a expression levels were significantly lower in Cr(VI)-transformed Cr-T cells. By using the mouse model, the expression levels of miR-199a were significantly decreased in blood samples and lung tissues of mice intranasally exposed to Cr(VI) for 12 weeks compared to the solvent exposure control. Overexpression of miR-199a inhibited tube formation and angiogenesis. C-X-C motif chemokine ligand 8 (CXCL8, IL8) levels were significantly higher in blood samples of Cr(VI)-exposed workers compared to normal workers, and forced expression of miR-199a in the cells suppressed IL8 levels. miR-199a suppression induced expression of hypoxia-inducible factor 1α (HIF-1α) and nuclear factor kappa B (NF-κB) p65 to increase IL8 expression. With animal experiment, the results showed that miR-199a overexpression inhibited tumor growth and angiogenesis through inhibiting IL8, HIF-1α and NF-κB p65 expression in vivo. These results show that miR-199a/IL8 pathway is important in Cr(VI)-induced carcinogenesis and angiogenesis.

1. Introduction

Cr(VI) is a known cancer-causing agent. Chromate compounds have broadly utilized in different ventures with its physio-substance properties, like flexibility, pliability, color and rigidity. The increased lung cancer risk is identified by workers exposed to Cr(VI) (IARC, 1990). The incidence of Cr(VI)-exposed workers has consistently increased in the epidemiological studies with the increase of lung cancer risk by 80-fold (Tsuneta et al., 1980). Lung cancer is the most common malignancies in human, accounting for 11.6% of all cancer occurrences, and is estimated to be 236,740 new lung cancer cases in the United States in 2022 (Siegel et al., 2022). Lung cancer is still the top cause of cancer deaths worldwide (18.4% of all cancer deaths), resulting in huge social burden and financial loss (Bray et al., 2018; Siegel et al., 2022). However, the exact mechanism of lung cancer occurrence induced by Cr(VI) remains elusive.

Recent study showed that microRNAs (miRNAs) were involved in Cr(VI)-induced malignant transformation (Chen et al., 2019; Humphries et al., 2016; Wang et al., 2021). MiRNAs are 18–24 nucleotide RNAs that are non-protein coding RNAs to regulate expression of many genes by targeting their mRNAs leading to the mRNA decay or inhibition of protein translation (Liu et al., 2011; Wang et al., 2019; Xu et al., 2013; Xu et al., 2012). In this study, miR-199a has been observed to be decreased in Cr-T cells compared to the human bronchial epithelial B2B cells. Furthermore, miR-199a expression was also significantly decreased in blood samples of Cr(VI)-exposing workers compared to...
non-exposing subjects. The mechanisms of miR-199a in Cr(VI)-induced tumorigenesis and angiogenesis remain to be elucidated.

IL8 is a chemical inflammatory factor that contributes to the development of cancer through inducing the proliferation, migration, invasion and angiogenesis of the tumor cells (He et al., 2013; Mantovani et al., 2008; Zlotnik, 2006). IL8 increases tumorigenicity and metastasis potency in xenograft and in vivo orthotopic models of various solid cancers, such as melanoma and bladder cancer, which improves the implantations of metastatic lesions (Bendre et al., 2005; Karashima et al., 2003). In human samples of Cr(VI) exposing workers and Cr-T cells, IL8 were overexpressed. However, the role of IL8 in Cr(VI)-induced carcinogenesis and lung tumorigenesis, remains unclear.

Cr(VI) is an established cause of lung cancer. In this study, we address following questions: (a) what role miR-199a plays in Cr(VI)-inducing carcinogenesis and angiogenesis; (b) what mechanism of miR-199a suppression is in inducing Cr(VI) carcinogenesis; (c) whether higher miR-199a/IL8 expression levels are associated with lung cancer development; (d) whether IL8 levels and miR-199a suppression levels are associated with Cr(VI) exposure of human population.

2. Materials and methods

2.1. Human blood and tumor samples

Blood samples were collected from non-exposed workers and chromium-exposed workers for several years and stored in the Tissue Bank of Zhengzhou University. None of the blood sample participants ever had chemotherapy or other major diseases. Blood samples were obtained from 20 non-exposed workers and 40 chromium-exposed workers in the Tissue Bank of Zhengzhou University. We removed red blood cells, platelets, and then separated plasma and peripheral blood monocytes (PBMCs). None of personal information is available to the investigators. The process has been approved by the Ethics Committee of Zhengzhou University.

The human lung cancer samples and adjacent normal tissue samples were obtained from the Biobank of the Affiliated Cancer Hospital of Zhengzhou University. All clinical samples were histologically classified and diagnosed by a clinical pathologist using the CoPath Anatomic Pathology System. For this study, no HIPPA-regulated patient information was available to investigators.

2.2. Cell culture and reagents

B2B cells were purchased from the ATCC (American Type Culture Collection, USA) and cultivated in DMEM (Gibco, USA). The Cr-T cells were malignant transformed by treated B2B cells for 6 months with 0.5 μM potassium dichromate (Sigma-Aldrich, USA) and tumor formation tests were conducted to validate malignant transformation status (He et al., 2013). Human lung cancer cell lines H1650, H1299, A549 and H1975 were obtained from ATCC and cultured in the medium RPMI-1640.

2.3. Immunoblotting

Total proteins were extracted by RIPA lysis buffer with protease inhibitors PMSF for 30 min on ice, and centrifuged at 13000 rpm, 4 °C for 15 min. The supernatants were gathered and protein concentrations were determined by BCA kit (Thermo Fisher Scientific, USA), then subjected to Western blotting. ECL Detection System was utilized for signal detection. Rabbit-anti-GAPDH and Rabbit-anti-HIF-1α were purchased from Bioworld Technology (Bloomington, USA), and Rabbit-anti-IL8 and Rabbit-anti-NF-κB p65 were purchased from Proteintech (Proteintech Group, USA).

2.4. RT-qPCR analysis

Total RNAs were extracted with Trizol Reagent as described in our previous study (Wang et al., 2022). The reverse transcription kit and the SYBR-Green Master Mix were used to detect expression levels of miR-199a, IL8, HIF-1α and NF-κB p65 (Vazyme, China). The housekeeping genes used in the study are U6 and GAPDH, respectively.

2.5. miRNA transfection

Cells have been cultivated in 6-well plates to reach 50%–60% confluence and transfected using Lipofectamine 3000 (Invitrogen, USA) in accordance with the directions from the manufacturer with 100 nM miR-199a mimic (200 nM miR-199a-inhibitor) or miR-NC mimic (miR-NC inhibitor) (Invitrogen, USA). Total proteins and RNAs were extracted from the cell of 6-well plates after 48–72 h transfection.

2.6. Luciferase activity assay

Cells were cultured in 24-well plates overnight and co-transfected with luciferase reporter plasmids (NF-κB p65 or VEGF) as well as Renilla luciferase reporter plasmid (internal control) using Lipofectamine 3000, and equal amounts of indicated miRNA mimic or inhibitor. After transfection for 24–48 h, dual-luciferase activities (Firefly and Renilla) were measured by a dual-luciferase assay kit (Promega, WI, USA) as described in our previous study (Wang et al., 2019).

2.7. Tube formation

The conditional medium was prepared by substituting normal culture medium for serum-reducing medium (1% FBS) from different cells. The serum-reduced medium were collected and kept for subsequent analysis after 24 h. HUVECs were grown in a complete medium and switched to 0.2% FBS basic medium for 24 h for testing tube formation activity. Then HUVECs were trypsinized and mixed with the same volume of the conditioned medium, seeded with 2 × 10⁵ cells/well on a Matrigel 96-well plate. For 6–12 h, the formation of the tube was observed, measured and photographed under microscope. Image J software was used to measure the total tube lengths of every well.

2.8. Chicken chorioallantoic membrane (CAM) Model

The fertilized chicken eggs (White Leghorn) have been incubated at 37 °C. These indicated Cr-T cells were trypsinized, counted in the serum-free medium and resuspended. Matrigel mixed the cellular suspensions at a 1:1 ratio and implanted them onto chorioallantoic membranes (CAMs) of chicken eggs. Results of tumor angiogenesis have been analysed after 9 days of implantation. CAMs have been removed and photographed from the tumor/matrigel plugs, the number of blood vessels as an angiogenesis index has been determined in three representative areas (1.5 mm²).

2.9. Cr(VI)-exposed mouse model by intranasal instillation

The 5–6 weeks old BALB/cJ mice were housed under standard animal laboratory conditions. Zinc chromate (ZnCrO₄) particles were prepared in sterile, pyrogen-free saline at an initial concentration of 1.0 mg/ml. Animals have been exposed to a ZnCrO₄ dose of 50 μl or saline once weekly intranasally as previous study (Bieaver et al., 2009), after 12 weeks of exposure to ZnCrO₄, blood samples were collected. PBMCs were isolated from blood samples, and total RNAs were extracted to detect the expression levels of miR-199a.

2.10. Tumor xenograft animal experiment

Female nude mice were housed under standard animal laboratory
conditions. For tumor growth testing, Cr-T cells with stable-expressed miR-199a and miR-NC have been injected into both sides of mice subcutaneously. When the tumors were visible, tumor sizes were measured with a vernier caliper every 2 days. The volumes of tumors were analyzed by the formulation of Volume\(=\frac{(\text{width}^2\text{mm}^2)\times\text{length}\text{mm}}{2}\). To test indicated protein and mRNA expression in animal tumor tissues, total proteins and RNAs were extracted for immunoblotting and qPCR. Tumors were fixed by formalin, paraffin-embedded, sectioned at 5 µm, and immunohistochemistry analysis in animal tissues were stained with anti-Ki67 and anti-CD31 antibodies (Proteintech Group, USA) and conducted as we described previously (Jiang et al., 2018).

2.11. Statistical analysis

All results have been obtained from at least three independent experiments and data analyzed via GraphPad Prism 7 software. Statistical evaluation for data analysis was determined by a t-test. The differences were considered to be statistically significant at \(P<0.05\).

3. Results

3.1. miR-199a expression levels are significantly lower in blood samples of Cr(VI)-exposed workers and mice, as well as in Cr(VI)-transformed (Cr-T) cells and human lung cancer cells

To figure out the potential role of miR-199a in Cr(VI)-exposed workers, we first tested expression levels of miR-199a in blood samples from Cr(VI)-exposed workers by qRT-PCR, and found that levels of miR-199a in human samples with Cr(VI) exposure were significantly downregulated (Fig. 1A). We also exposed BALB/cJ mice to ZnCrO\(_4\) for 12 weeks to collect peripheral blood mononuclear cells (PBMCs) of blood samples, and showed that expression levels of miR-199a were much lower in Cr(VI)-exposed group compared to the control (Fig. 1B). Furthermore, the expression levels of miR-199a in Cr(VI)-transformed (Cr-T) cells were greatly decreased compared to those in B2B cells (Fig. 1C). We subsequently tested miR-199a expression levels in lung cancer cells, and showed that miR-199a levels in human lung cancer cells (H1650, H1299, A549, H1975) were also greatly decreased compared to those in B2B cells (Fig. 1D). Additionally, miR-199a expression levels were downregulated in lung cancer cells by analyzing GSE datasets (GSE74190, GSE135918, GSE15008, GSE19945) (Fig. 1E). In line with the study mentioned above, miR-199a level may function as a potential biomarker for Cr(VI)-induced exposure.
Fig. 2. miR-199a inhibited tube formation and angiogenesis. (A) Representative tube pictures and quantification of HUVEC cultivated with conditioned medium on Matrigel-coated plates. (B) Cells were implanted into the CAM, and miR-199a overexpression inhibited angiogenesis. Top: representative pictures from each group; bottom: relative angiogenesis activities. Data were presented as mean ± SD of three independent experiments. * *Indicated significant difference at P < 0.01.

Fig. 3. IL8 was upregulated in human samples with Cr (VI)-exposure and Cr-T cells, and miR-199a attenuated the expression levels of IL8. (A) IL8 was upregulated in human blood samples with Cr (VI) exposure. (B) The protein and RNA expression levels of IL8 were identified using Western blotting and RT-PCR, and IL8 expression was upregulated in Cr-T cells. (C) miR-199a decreased the expression of IL8 at the protein levels, whereas anti-miR-199a inhibitor increased the expression of IL8. (D) miR-199a and IL8 levels showed inversely correlated each other. Data were presented as mean ± SD of three independent experiments. * *, # # Indicated significant difference at P < 0.05; * * *, # # # Indicated significant difference at P < 0.01.
3.2. miR-199a forced expression inhibits tube formation and angiogenesis

As miR-199a is downregulated in Cr-T cells, we wonder if miR-199a may have biological functions in tumor angiogenesis and cell transformation. Angiogenesis is the key process for tumor development (Viallard and Larrivee, 2017; Wang et al., 2021), the effect of overexpression of miR-199a in angiogenesis was assessed both in vitro and in vivo. Firstly, we studied the tube formation of the Cr-T-miR-199a cells using HUVEC cells, and found the tube formation activities were significantly inhibited compared to Cr-T-miR-NC cells (Fig. 2A). We applied the chicken CAM assay experiment for studying angiogenesis in vivo, which showed that overexpression of miR-199a in Cr-T cells inhibited angiogenesis compared to control cells (Fig. 2B). Thus, miR-199a inhibited tube formation and angiogenesis induced by Cr-T cells.

3.3. IL8 expression is upregulated in human blood samples with Cr (VI) exposure and in Cr-T cells, and miR-199a overexpression attenuated the expression levels of IL8

We firstly tested expression levels of IL8 in blood samples of Cr (VI)-exposed workers by qRT-PCR, and showed that IL8 expression levels were upregulated in the human examples with Cr (VI) exposure (Fig. 3A). To test whether miR-199a may inhibit IL8 expression levels, we further analyzed expression levels of IL8 in Cr-T and B2B cells by utilizing immunoblotting and RT-PCR, showing that IL8 levels were greatly upregulated in Cr-T cells (Fig. 3B). In addition, overexpression of miR-199a decreased IL8 expression at the protein level, while inhibition of miR-199a increased the expression of IL8 (Fig. 3C). Moreover, miR-199a and IL8 levels were inversely correlated with each other (Fig. 3D).

3.4. miR-199a overexpression inhibits the HIF-1α/IL8 pathway, which is important in tube formation

Hypoxia-inducible factor 1α (HIF-1α) mediates adaptive responses to changes in the oxygenation of tissues (Jiang et al., 1997). HIF-1α has been reported to regulate genes that encode tumor metabolism, neo-vascularisation, drug strength, invasion, autophagy and cell survival-related proteins (Ge et al., 2018). Multiple malignant tumors have been found to have highly expressed HIF-1α protein levels. The higher expression levels of HIF-1α were detected in the cells when miR-199a expression was suppressed and with the transfection of the anti-miR-199a inhibitor, whereas lower HIF-1α expression levels were observed after the treatment of cells using miR-199a (Fig. 4A). HIF-1α was suggested to activate VEGF expression by binding to the VEGF promoter region with the hypoxia response element. In order to determine if miR-199a inhibits VEGF expression through HIF-1α binding site, we analyzed the effects of miR-199a on VEGF reporter plasmid and
Fig. 5. miR-199a/HIF-1α/IL8 pathway regulated tube formation. (A-B) miR-199a inhibited tube formation activity, whereas overexpression of IL8 or HIF-1α reversed tube formation of HUVECs inhibited by miR-199a. Data were presented as mean ± SD of three independent experiments. * * Indicated significant difference at P < 0.01.

Fig. 6. miR-199a functioned as a tumor suppressor by inhibiting NF-κB p65/IL8 pathway. (A) In Cr-T cells, NF-κB p65 expression was induced. (B) NF-κB p65 protein and mRNA levels were determined by Western blotting and qRT-PCR analysis with the transfection of miR-NC/miR-199a or miR-NC-inhibitor/miR-199a-inhibitor in Cr-T cells. (C) miR-199a reduced NF-κB p65 luciferase activities, whereas the p65 luciferase activities were increased with anti-miR-199a inhibitor. (D) Overexpression of NF-κB p65 increased IL8 expression, whereas NF-κB p65 knockdown decreased IL8 expression. (E) We found a positive correlation between p65 and IL8 levels using the lung cancer database (http://www.bioinfo-zs.com/luadexpress/). Data were presented as mean ± SD of three independent experiments. * * Indicated significant difference at P < 0.05; * * * Indicated significant difference at P < 0.01.
showed that miR-199a inhibited VEGF reporter activities in Cr-T cells, which conveyed that miR-199a inhibits VEGF expression through HIF-1α (Fig. 4B). Furthermore, overexpression of HIF-1α induced IL8 expression; and HIF-1α downregulation decreased IL8 expression (Fig. 4C). In addition, we showed a positive correlation between HIF-1α and IL8 levels in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) clinical samples using the ENCORI database (https://starbase.sysu.edu.cn/) (Fig. 4D). Thus, these results showed that miR-199a inhibited HIF-1α/IL8 pathway in Cr(VI)-induced carcinogenesis as well as its association with lung cancer development. Furthermore, ectopic expression of IL8 or HIF-1α partially reversed miR-199a-inhibited tube formation activities (Fig. 5A-B). These results suggested that miR-199a inhibited HIF-1α/IL8 pathway in Cr(VI)-induced angiogenesis.

3.5. miR-199a functions as a tumor suppressor by inhibiting NF-κB/IL8 pathway

Our previous study suggested that NF-κB signaling is activated in Cr(VI)-transformed cells (He et al., 2013). The emergent pathogenic element in a range of human malignancies were the dysfunction of NF-κB signaling. The NF-κB p65 levels in Cr-T and B2B cells were then tested by immunoblotting and qRT-PCR (Fig. 6A). We further found that the NF-κB p65 protein levels in Cr-T cells were decreased with miR-199a overexpression, whereas inhibition of miR-199a increased NF-κB p65 expression (Fig. 6B). Then, the cells were transfected with NF-κB p65 luciferase vectors with miR-NC/miR-199a or miR-NC-inhibitor/miR-199a-inhibitor, showing that the luciferase activities of NF-κB p65 were decreased with miR-199a overexpression, and that miR-199a-inhibitor increased NF-κB p65 luciferase activities (Fig. 6C). NF-κB p65 also had an effect on the mRNA and protein expression levels of IL8, as Fig. 6D suggested, overexpression of p65 significantly induced IL8 expression, whereas p65 knockdown reduced IL8 expression. In addition, we showed a positive correlation between p65 and IL8 levels using the lung cancer database (http://www.bioinfo-zs.com/luadexpress/) (Fig. 6E). Thus, our results suggested that NF-κB p65 is the upstream transcription factor of IL8, and that miR-199a acted as a tumor suppressor by inhibiting NF-κB/IL8 pathway.

3.6. miR-199a overexpression inhibits tumor growth

To investigate the impact of miR-199a on tumorigenesis, Cr-T cells were treated with lentivirus carrying miR-199a. Stable cell lines expressing miR-NC were used as control. Cells were injected subcutaneously into the two flanks of nude mice, and tumor sizes were measured after 14 days of cell injection. Overexpression of the miR-199a in the cells significantly inhibited tumor development compared to the control group (Fig. 7A). The xenografts were cut off and weighed after 29 days. In miR-199a overexpression group, tumor size and weight were significantly inhibited compared to the miR-NC group (Fig. 7B and C). In addition, the protein levels of IL8, NF-κB p65 and HIF-1α in the miR-199a group were lower than the levels from miR-NC cells, which
confirmed the negative correlation of miR-199a with IL8, NF-κB p65 or HIF-1α levels in vivo (Fig. 7D). The mRNA expression levels of IL8, NF-κB p65 and HIF-1α were examined by qRT-PCR in tumor tissues, and miR-199a forced expression also inhibited IL8, NF-κB p65 and HIF-1α expression (Fig. 7E). IHC staining analysis revealed that miR-199a overexpression significantly decreased the expression levels of proliferative biomarker Ki67 and angiogenesis biomarker CD31, suggesting that miR-199a inhibited tumor growth and angiogenesis in vivo (Fig. 7F).

3.7. miR-199a, IL8, NF-κB p65 and HIF-1α levels are altered in human lung cancer tissues and correlated with the overall survival (OS) of lung cancer patients

In this study, we analyzed the expression levels of miR-199a in 24 pairs of NSCLC and normal tissues, and found that miR-199a levels in NSCLC tissues were downregulated compared to normal tissues (Fig. 8A). Besides, expression levels of IL8, NF-κB p65 and HIF-1α were examined in the same clinical samples, which showed that IL8, NF-κB p65 and HIF-1α expression levels in the tumor tissues were higher than the normal tissues (Fig. 8B). High expression levels of miR-199a in the lung cancer tissues showed better overall survival prognosis in lung cancer patients using Kaplan Meier plotter (http://kmplot.com/examination/) (Fig. 8C, p = 0.0099). Furthermore, Kaplan Meier plotter analysis was also used to determine the correlation of overall survival (OS) with levels of IL8 (p = 1.9e-10), NF-κB p65 (p = 0.0075) and HIF-1α (p = 0.01) in lung cancer tissues, the results showed that higher expression levels of IL8, NF-κB and HIF-1α in tumors showed poor overall survival prognosis in lung cancer patients (Fig. 8D). In conclusion, miR-199a/IL8 pathway is important in Cr(VI)-initiated carcinogenesis, cancer development and angiogenesis.

4. Discussion

Hexavalent chromium [Cr(VI)] is classified as a carcinogenic reagent in human. Since chromium is widely used industrially, a large amount of Cr(VI) has been found to be released into the environment. Due to its commercial use in welding, chrome pigments, leather tanning, and ferrochrome industries, Cr(VI) is exposed to multiple occupational environments (Fishbein, 1981; Malsch et al., 1994). Cr(VI) is also an industrial waste and air pollution component. While earlier studies showed that DNA breaks, chromosome instability as well as other genomic alterations are induced by exposure to acute Cr(VI), we proposed the occurrence of Cr(VI)-induced miRNA dysregulation as the mechanism of Cr(VI) carcinogenesis in this study.

MiRNAs are one of the most interesting fields of research because they regulate a large proportion of gene expression in cells (He et al., 2019; Jiang et al., 2018; Wang et al., 2019). MiRNAs are recently found stable in serum or plasma cell-free forms and can be released through regular cells and/or tumors into the blood system. Expression levels of various miRNAs in the blood were found to be altered in several cancers (Jin et al., 2020; Li et al., 2019; Wang et al., 2022). We analyzed the expression levels of miR-199a in blood samples for non-exposed workers/mice and subjects exposed to Cr(VI). Interestingly, we found that the circulating levels of miR-199a in workers/mice exposed to Cr(VI) were significantly reduced. These results strongly suggested that miR-199a can be used as a new biomarker for evaluating Cr(VI)-exposition.

The recruitment and activation of neutrophils and granulocytes at the site of inflammation is regulated by IL8 (CXCL8), which is
recognized as a prototype chemical of the CXC family (Fousek et al., 2020; Waugh and Wilson, 2008). In physiological conditions, IL8 is almost undetectable but rapidly induced, which was similar to pro-inflammatory cytokines (TNFα) and interleukin-1b (IL-1b) (Hoffmann et al., 2002). The mechanism of IL8 signals in tumorigenesis and progression was extensively explored. IL8 is known to induce angiogenesis and to increase the tissue re-modeling matrix metalloproteinase (MMP) expression (Azenshtein et al., 2005; Kim et al., 2001). Higher IL8 levels show a higher risk of cancer and a poor prognosis (Balaosui et al., 2014; Pine et al., 2011). Our data in this study revealed the higher-expression levels of IL8 in workers exposed to Cr(VI), which was consistent with upregulation of IL8 in Cr-T cells. Our results show IL8 is an important regulator in Cr(VI)-induced carcinogenesis.

In the tumor micro-environment, hypoxia is prevalent due to physical and functional changes in blood vessels and the increase in oxygen intake due to rapid tumor cell growth (Harris, 2002). HIF-1α controls the homeostasis of oxygen. HIF-1α and hypoxia are the main factors for angiogenesis (Semenza, 2001). These factors control cell invasion and metastasis, which eventually determines tumor cell aggression (Koh et al., 2011). In a range of human malignancies, dysfunction of NF-κB signaling was the emerging pathogenic element. To date, a number of efforts have been made to investigate the anti-tumor potential for targeting of NF-κB p65 pathway (Erstad and Cusack, 2013). Interestingly, HIF-1α and NF-κB p65 were regulated as transcription factors for IL8 expression. It is interesting to study whether Cr(VI)-induced carcinogenesis could be involved in HIF-1α and NF-κB p65 or other potential factors. We found that miR-199a inhibited tumor growth and angiogenesis with the animal experiment in vivo by regulating HIF-1α, NF-κB p65 and IL8 expression. Interestingly, Kaplan Meier’s analysis plotter showed that the overall survival correlation of miR-199a, HIF-1α, NF-κB p65 and IL8 expression levels in the tissues of lung cancer, which indicates pivotal role of miR-199a/IL8 pathway in Cr(VI)-initiated carcinogenesis.

5. Conclusion

Cr(VI) is a common and well-known environmental carcinogen, that causes lung and other cancers in humans, the mechanism of Cr(VI)-induced carcinogenicity is not clearly understood. In this study, our new results showed chronic exposure to Cr(VI) downregulated miR-199a expression in the cells, animal and human subjects; and miR-199a downregulation is important in mediating Cr(VI)-initiated carcinogenesis and angiogenesis. Lower miR-199a levels were associated with higher IL8 expression levels in lymphocytes of human samples. The miR-199a suppression increased transcription factors HIF-1α and NF-κB p65 expression levels to induce IL8 expression. The levels of miR-199a, IL8, HIF-1α, and NF-κB p65 expression may be used as potential novel biomarker(s) of Cr(VI) exposure or/and new target for cancer prevention in the future. However, it is still unknown whether Cr(VI) exposure may cause epigenetic changes to downregulate miR-199a expression.

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CRediT authorship contribution statement

Lin Wang: Methodology, Data generation, Writing, Investigation.
Zhi-Hao Zhou, Yun-Xia Xie, Wen-Jing Liu: Data generation and analysis.
Rui-Xiang Zhang, Nan Jiang: Methodology, Software.
Jian-Ge Qiu, Ming-Liang He, Bing-Hua Jiang: Conceptualization, experimental design, manuscript preparation and editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Data Availability

No data was used for the research described in the article.

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