Peer Review File

Article Information: https://dx.doi.org/10.21037/atm-22-3293

Reviewer A

Comment 1: The authors have presented an interesting topic that explores a potentially novel miRNA-mediate mechanism in BPD injury. Although interesting, the authors did not present unequivocal evidence that up regulation of miR-495 leads to a more severe outcome in BPD.
The authors present initially miRNA screening data in BPD patients to uncover that miR-495 is a highly unregulated target and is paired with the up regulation of miR-495 predicted genes. Although an important first step in presenting the argument for MiR-495's involvement, there is no evidence that this up regulation causes an injury phenotype.
To prove a miR-495 mediated mechanism, the authors must include miR-495 targeted knock-down or up regulating experiments to specifically modify those pathways in vivo. Will blocking miR-495 up regulation, in vivo, decreased the BPD injury? Similarly, will targeting miR-495 targets, provide protection or reversal of the BPD injury.
In its current state, the manuscript only identifies a pathway that is modified by a BPD injury, however, does not suggest if modification of this pathway may lead to a therapeutic or physiologic benefit. More information needs to be provided to demonstrate that miR-495 is involved in the process of injury, rather than just associated.
Reply 1: Thank you for your valuable comments on our manuscript. A number of miRNAs have been reported to play important roles in BPD. We aims to explore the physiological function of miR-495 in BPD. We found that abnormal overexpression of miR-495 in peripheral blood of BPD premature infants compared with control group and verified the binding ability of miR-495 to its predicted target region, 3'UTR of NEDD4L *in vitro*. In LPS-induced BPD neonatal rat model, the decreased NEDD4L expression led to the upregulation of ENaC, the key regulator of lung fluid clearance during perinatal period. We speculated that the aberrant overexpression of miR-495 might be involved in BPD by targeting NEDD4L/ ENaC. However, the limitations of our experiments are also obvious. The current findings could not demonstrate that miR-495 overexpression causes more severe BPD injury through the NEDD4L/ENaC pathway and its specific molecular mechanism remains to be further illustrated. Related experiments on inhibiting miR-495 overexpression in *vivo* need to be performed to prove the miR-495 mediated mechanism in BPD. We have added this in the future perspectives in the discussion part (See Page 14, Line 299-301).
Changes in the text: Further experiments on inhibiting miR-495 overexpression *in vivo* need to be performed to prove the miR-495 mediated mechanism in BPD.

Reviewer B

Thank you very much for reviewing our manuscript. We apologize for the typos/ grammatical errors, which have been revised in our resubmitted manuscript. Thanks for your correction. More responses are listed below.

Comment 1: Abstract: Line 15 – typo: “BPD has not yet been fully understood”.
Reply 1: We have corrected the typo and modified our text as advised (See Page 2, Line 24)**.**
Changes in the text: However, the physiological function of miR-495 in BPD has not yet been fully understood.

Comment 2: Abstract: Line 27 – awkward wording makes it unclear if the pups or the mom’s have BPD. I assume it’s the pups, but that’s not what you wrote.
Reply 2: Thank you for your reminder and we have changed the “rats’ pups” to “rat pups” as advised (See Page 2, Line 36)**.**
Changes in the text: Meanwhile, the miR-495 expression was also increased in lungs of rat pups with BPD at postnatal day (P) 3 compared to the control group.

Comment 3: Introduction: Line 47 to 49: several grammatical errors, should read: “revealed that the imbalance of lung luminal fluid is involved in the development of BPD through the largely unknown mechanisms [1, 3]. Previous studies have found that increased ENaC activity was responsible for alveolar lipid depletion and hindered lung function”
Reply 3: We have modified the sentences as advised (See Page 3, Line 55-58)**.**
Changes in the text: Recently, a growing number of studies have revealed that the imbalance of lung luminal fluid is involved in the development of BPD through the largely unknown mechanisms (1,3). Previous studies have found that increased ENaC activity was responsible for alveolar lipid depletion and hindered lung function (4).

Comment 4: Introduction: Line 55: that is not the correct usage of the word “successively”. I’m not sure what you mean.
Reply 4: We have removed the word in the sentence (See Page 3, Line 64)**.**
Changes in the text: The association of altered expression of multiple miRNAs with impeded alveolarization has been reported.

Comment 5: Introduction: Line 57: most grammatical errors, should read: “MiR-495 was found to have multiple regulatory functions in a number
Reply 5: We have corrected errors in the text as advised (See Page 3, Line 66)**.**
Changes in the text: MiR-495 was found to have multiple regulatory functions in multiple lung diseases, including lung cancer, pulmonary hypertension, acute lung injury and chronic asthma.

Comment 6: Introduction: Line 63: “validated” is not the right word. You validate a procedure, a method, a model. Maybe you mean to say that you “found”…
Reply 6: We have changed the word " validated " to "found" in the sentence as advised (See Page 4, Line 72)**.**
Changes in the text: In the present study, we found abnormal overexpression of miR-495 in the peripheral blood of BPD premature infants.

Comment 7: Methods: Was the human sample portion of this study an IRB approved study or

something similar? How were the samples processed?

Reply 7: The human sample portion of our study was approved by the ethics committee of Shanghai Children's Hospital, Shanghai Jiao Tong University (No.2015RY009-F01). The details of the sample processing have been described in our previous study and we have quoted the study in our article. The premature infant (<32 weeks gestational age) diagnosed with BPD had radiographic confirmation of persistent parenchymal lung disease and still needs the premise that auxiliary oxygen (invasive intermittent positive pressure ventilation (IPPV), nasal continuous positive airway pressure (N-CPAP)/non-invasive positive pressure ventilation (NIPPV), nasal cannula, Hood O2) at 36 weeks postmenstrual age with one of the following FiO_2 ranges/oxygen levels/O2 concentrations for ≥3 consecutive days to maintain arterial oxygen saturation in the 90–95% range. The non-BPD premature infants had no history of oxygen inhalation or no long-term history of oxygenation and therefore did not develop BPD. Their underlying diseases were mainly premature or accompanied by neonatal respiratory distress syndrome (NRDS). There were no perinatal infections among these infants, and the admission time was within 12 h after birth. After obtaining the informed consent of the guardian of the patients and signing a written document, we collected the peripheral blood of the premature infants as samples for RT-PCR. The time points of peripheral blood sample collection were 28 days after continuous oxygen use in the BPD group, and the control group was also at the same gestational age for no more than 3 days. We added this in the manuscript (See Page 4, Line 83-84; Page 5, Line 90-92)**.**

Changes in the text: The peripheral blood samples of 6 BPD premature infants and 6 age-matched non-BPD preterm infants were collected as described in our previous study (14).

This study was approved by the ethics committee of Shanghai Children's Hospital, Shanghai Jiao Tong University (NO.2015RY009-F01) and informed consent was taken from all the patients.

Comment 8: Methods: How was the matching done. Were the controls 3 day old babies of the same gestational age? Were they the same chronologic age? the same corrected age? A 23 week baby who is now 28 days old is very different from a 3 day old of any gestational age. How do you know the differences aren't related to age? You need to explain this a lot more and if the samples aren't really matched, then this data is not useful.

Reply 8: We have compared the general clinical characteristics in the two groups, including gestationally corrected age, weight at the time of sample collection and sex. No statistically significant differences were found. We added this in the part of Methods (See Page 4-5, Line 86-89)**.**

Changes in the text: No statistically significant differences were found in the comparison of general clinical characteristics (postmenstrual age, weight at the time of sample collection, and sex) in the two groups (Table I).

Comment 9: Methods: Line 74 – 75: the first sentence is not grammatically correct. It's unclear what was from your previous study.

Reply 9: We collected the peripheral blood samples as described in our previous studies. We modified the sentences as advised (See Page 4, Line 83-84)**.**

Changes in the text: The peripheral blood samples of 6 BPD premature infants and 6 age-

matched non-BPD preterm infants were collected as described in our previous study (14).

Comment 10: Methods: Line 75: what clinical characteristics were compared. You need to include a demographics table showing the characteristics of both groups.
Reply 10: We compared the terms of postmenstrual age, weight, and sex between the two groups. We added a table in the part of Methods (See Page 4-5, Line 86-89)**.**
Changes in the text: No statistically significant differences were found in the comparison of general clinical characteristics (postmenstrual age, weight at the time of sample collection, and sex) in the two groups (Table I).

Comment 11: Methods: Line 76: you mean "characteristics" not "characters"
Reply 11: We have changed the word " characters " to " characteristics " in the sentence as advised (See Page 4-5, Line 86-89)**.**
Changes in the text: No statistically significant differences were found in the comparison of general clinical characteristics (postmenstrual age, weight at the time of sample collection, and sex) in the two groups (Table I).

Comment 12: Methods: Line 79: I'm not sure what you mean by "according to previous study" this is incorrect grammar
Reply 12: We have removed these words and modified the sentence (See Page 4, Line 84-86).
Changes in the text: The time points of peripheral blood sample collection were 28 days after continuous oxygen use in the BPD group, and approximately 3 days after birth in the control group.

Comment 13: Animal model: Line 88: what does EP stand for?
Reply 13: EP stands for "Eppendorf tube". We added the full form of EP in the text (See Page 5, Line 101-103).
Changes in the text: Lungs were dissected free of heart and trachea and placed into Eppendorf tubes for further investigation.

Comment 14: Animal model: H&E staining: Were the lungs insuflated prior to fixation? That is the correct way to look at lung pathology.
Reply 14: The lungs were insufflated prior to fixation in our study. We added it in the text as advised (See Page 6, Line 118)**.**
Changes in the text: Lung tissue was insufflated and fixed with 4% buffered paraformaldehyde.

Comment 15: Results: Line 167 – you don't mean "notice" you mean "knowledge"
Reply 15: We have changed the word "notice" to "knowledge" in the sentence as advised (See Page 9, Line 182-184)**.**
Changes in the text: To our knowledge, miR-495 as formally reported is involved in regulating lung development and various lung diseases by targeting a series of downstream signaling pathways.

Comment 16: Results: Line 172 – the sentence "The expression of miR-495 were measured

by RT-qPCR" is repetitive. You don't need it.
Reply 16: We have removed this sentence as advised (See Page 9, Line 185-187).
Changes in the text: To investigate the role of miR-495 in BPD, we reanalyzed miR-495 expression in the peripheral blood samples of 6 BPD premature infants and 6 age-matched non-BPD preterm infants by using RT-qPCR.

Comment 17: Results: What samples were the results described under "MiR-495 directly targets NEDD4L by interaction with the 3'UTR.' Done on. You need to be more clear.
Reply 17: A549 cells were used in our study for dual-luciferase reporter assay. We have modified the sentence as advised (See Page 9, Line 190).
Changes in the text: MiR-495 directly targets NEDD4L by interaction with the 3'UTR in A549 cells.

Comment 18: Results: Line 199 – you mean "previously"
Line 200 – you mean "shown" not "showed"
Line 209 – you mean "compared" not "compaed"
Reply 18: We have corrected these errors as advised (See Page 10, Line 214-217; Page 11, Line 223-225).
Changes in the text: Previous studies have shown that NEDD4L overexpression inhibits the activation of the epithelial Na+ channel (ENaC), which was a key regulator of fluid balance in lung development and involved in the transition from lung fluid secretion to fluid absorption. The results of the western blot showed that NEDD4L expression in the LPS-treated group was reduced compared with that in the control group.

Comment 19: Discussion: Line 226 – Not sure what you mean by "significant dynamic changed"
Reply 19: We meant that the miR-495 expression was different at different stages of rat lung development. It has been reported that the miR-495 expression was upregulated from canalicular to saccular stages and downregulated from saccular to alveolar stages in rat lung development. We have modified the sentence as advised (See Page 11-12, Line 241-244).
Changes in the text: Yang and his colleagues found that miR-495 expression underwent significantly dynamic changes in rat lung development. The expression of miR-495 was upregulated from canalicular to saccular stages and downregulated from saccular to alveolar stages.

Comment 20: Discussion: Line 228 – 230 – Should be something like: "The above findings suggested that the altered expression of miR-495 might participate in the regulation of lung development in BPD."
Reply 20: We have modified the sentence as advised (See Page 12, Line 244-246).
Changes in the text: The above findings suggested that the altered expression of miR-495 might participate in the regulation of lung development in BPD.

Comment 21: Lines 230- 232 – Should be something like: "Then, we detected increased expression of miR-495 in peripheral blood of BPD infants by RT-qPCR, which was.."

Reply 21: We have modified the sentence as advised (See Page 12, Line 246-247).
Changes in the text: Then, we detected increased expression of miR-495 in peripheral blood of BPD infants by RT-qPCR, which was opposite to the findings in normal rat lung development.

Comment 22: Line 237 – should be something like "plays an important role in…"
Reply 22: We have modified the sentence as advised (See Page 12, Line 251-253).
Changes in the text: NEDD4L (also known as NEDD4-2), a ubiquitin-protein ligase in the NEDD4L family, can regulate a fair amount of membrane proteins and plays an important role in maintaining the homeostasis of cells.

Comment 23: Line 247-249 – This sentence is completely wrong. It reads like you used google translate to write it. "BPD is featured with the retardation of lung development at late stage and led to simplified alveolar structure and abnormal vascular development[1, 2]" you need to re-write it.
Reply 23: We have rewritten this sentence as advised (See Page 12, Line 262-263).
Changes in the text: The pathgnomic of BPD is characterized by impaired alveolarization and dysregulated vascularization.

Reviewer C

Comment 1: Line 27 – rats' pups may be changed to rat pups
Reply 1: We have changed "rats' pups" to "rat pups" in the sentence as advised (See Page 2, Line 35-36).
Changes in the text: Meanwhile, the miR-495 expression was also increased in lungs of rat pups with BPD at postnatal day (P) 3 compared to the control group.

Comment 2: Lines 29-31: The explanation is not clear. Please elaborate. IF data Opposite means to what –to human data or to WB/PCR data?
Reply 2: We meant that the changes in NEDD4L expression were opposite to those in ENaC expression. We have rewritten this sentence to make it clear (See Page 2, Line 38-41).
Changes in the text: The IF staining result showed that the NEDD4L expression was decreased, and ENaC expression was increased in the LPS-induced BPD rat model, which was consistent with abnormal changes in alveolar structure.

Comment 3: Line 47 – remove 'is involved'.
Reply 3: We have rewritten this sentence based on the suggestions of you and the other reviewers (See Page 3, Line 55-57).
Changes in the text: Recently, a growing number of studies have revealed that the imbalance of lung luminal fluid is involved in the development of BPD through the largely unknown mechanisms.

Comment 4: Line57 – change to "MiR-495 was found to have multiple regulatory functions".
Reply 4: We have modified the sentence as advised (See Page 4-5, Line 66-68).
Changes in the text: MiR-495 was found to have multiple regulatory functions in multiple

lung diseases, including lung cancer, pulmonary hypertension, acute lung injury and chronic asthma.

Comment 5: Lines 74-75. Please quote your study which was already published (PMID: 31948520). How is that study different from the current study with respect to the BPD patient data?
Reply 5: Numerous studies have shown that miRNAs play promotional or protective roles in the development of BPD by targeting distinct pathways. Our previous study found that the increased expression of adrenomedullin regulated by miR-574-3p can protect premature infants with BPD through antioxidation and anti-inflammation. This study suggested that the overexpression of miR-495 may contribute to the development of BPD by targeting NEDD4L/ ENaC implying an imbalance in lung fluid clearance. We quoted the previous study in our text (See Page 4, Line 83-84).
Changes in the text: The peripheral blood samples of 6 BPD premature infants and 6 age-matched non-BPD preterm infants were collected as described in our previous study (14).

Comment 6: Line 86 – change to "Pregnant rats".
Reply 6: We have corrected the errors as advised (See Page 5, Line 100-101).
Changes in the text: Pregnant rats injected with sterile and endotoxin-free saline (5ul) were used as control.

Comment 7: Line 87 – change 'killed at' to 'sacrificed or euthanized at'.
Reply 7: We have modified the sentence as advised (See Page 5, Line 101).
Changes in the text: Newborn rats were sacrificed at postnatal day (P) 3.

Comment 8: Line 88 – Write the full form of EP.
Reply 8: EP stands for "Eppendorf tube". We have added the full form of EP in the text as advised (See Page 5, Line 101-103).
Changes in the text: Lungs were dissected free of heart and trachea and placed into Eppendorf tubes for further investigation.

Comment 9: Line 90 – Include the approval number
Reply 9: We added the approval number in the text as advised (See Page 5, Line 103-105).
Changes in the text: Experiments were performed under a project license (NO.2018009) granted by the ethics committee of Shanghai Children's Hospital, Shanghai Jiao Tong University, in compliance with its guidelines for the care and use of animals.

Comment 10: Line 93 – Is there a specific reason for choosing A549 cells? It is a cancer cell line and usually lung researchers would not use this for general lung-associated studies. Have you tried any other cell lines like MLE-12, MLE 15 or similar ones? I would strongly recommend including data from other cell lines as data from A549 is usually not acceptable.
Reply 10: The A549 cells were used for dual-luciferase reporter assay in our study for its high transfection efficiency. Meanwhile, the A549 cells are originated from human alveolar cell adenocarcinoma and possess many characteristics of native type II cells. Therefore, we used

A549 cells in our study.

Comment 11: Line 160 – change to 'indicate statistical significance'.
Reply 11: We have modified the sentence as advised (See Page 8, Line 174-175).
Changes in the text: $P<0.05$ was considered to indicate statistical significance.

Comment 12: Lines 163-166 – Are the results submitted to the GEO database published? If then please quote the article. How significant was the difference in the array between the normal and BPD serum samples as I see both in the red range?
Reply 12: The results submitted to the GEO database have been published and we have quoted the article in our text as advised (See Page 9, Line 179-181). Some of the results can be found in Figure 1 (See Page 9, Line 182). Although both are red, the shades are different, indicating a difference between the two groups.
Changes in the text: In the previous study, we compared and analyzed the differentially expressed miRNAs in peripheral blood of BPD premature infants by microarray expression profiling and the results have been submitted to the GEO database (GSE108755) (14).

Comment 13: Line 167 – change 'a various of miRNAs' to 'various miRNAs'
Reply 13: We have modified the sentence as advised (See Page 9, Line 182).
Changes in the text: To date, various miRNAs were differentially expressed as shown in Figure 1A.

Comment 14: Line 168 – change to 'reported is involved…'
Reply 14: We have modified the sentence as advised (See Page 9, Line 182-184).
Changes in the text: To our knowledge, miR-495 as formally reported is involved in regulating lung development and various lung diseases by targeting a series of downstream signaling pathways.

Comment 15: Line 188 – change to 'for further studying..'
Reply 15: We have modified the sentence as advised (See Page 10, Line 203-204).
Changes in the text: …we established the rat model of BPD for further studying miR-495 regulatory downstream signaling *in vivo*.

Comment 16: Lines 190-191 – 'Almost all the rats of both groups survived to the end of the experiment'. This sentence must be changed. Either modify to 'All animals survived. If not mentioned as 1 out of 6' or whatsoever number died in this group.
Reply 16: We have modified the sentence as advised (See Page 10, Line 205).
Changes in the text: All the animals survived.

Comment 17: Line 199 – change to Previous studies.
Reply 17: We have modified the sentence as advised (See Page 10, Line 214-217).
Changes in the text: Previous studies have shown that NEDD4L overexpression inhibits the activation of the epithelial Na+ channel (ENaC), which was a key regulator of fluid balance in lung development and involved in the transition from lung fluid secretion to fluid absorption.

Comment 18: Line 209 – correct the spelling of compared.
Reply 18: We have corrected the error (See Page 11, Line 223-225).
Changes in the text: The results of the western blot showed that NEDD4L expression in the LPS-treated group was reduced compared with that in the control group (Figure 4B).

Comment 19: Line 405 – Why (B: BPD group, C: control group). B or C is not in the picture.
Reply 19: We have removed these words as advised (See Page 20, Line 427-428).
Changes in the text: Some of them were showed in this figure.

Comment 20: Is BAL analyzed for these pups? What is the cell count and protein? Include BAL data would be good.
Reply 20: Thank you very much for your suggestion. Due to the difficulties of operation, we did not perform the BAL analysis in this study.

Comment 21: How did you study the lung-fluid balance? Did you quantify the fluid absorption/clearance in the lung? Including the lung wet/dry weight ratio is highly recommended as NEDD4L and ENaC are known to have a role in fluid absorption and there are affected in BPD.
Reply 21: The process of lung fluid clearance after birth is the absorption of fluid from the alveolar lumen into the interstitium and then through pulmonary blood circulation and lymphatic circulation. This process helps prevent excessive buildup of fluid while ensuring adequate humidification of the alveolar surface[1,2]. ENaC plays a key role in postnatal lung fluid clearance, mainly by transferring fluid from the alveolar lumen to the interstitium [1-4]. BPD is a chronic lung disease of prematurity, in which lung edema due to interstitial fluid accumulation is one of the main pathological features[1,5,6]. The above findings suggest that overexpression of ENaC in BPD could cause an increase in fluid transfer from the alveolar lumen to the interstitium, which might lead to excessive absorption of lung epithelial fluid and increased interstitial edema. Thus, the lung wet/dry weight ratio could merely allow a comparison of lung tissue fluid content between the BPD and non-BPD groups, but it was not possible to assess whether ENaC overexpression promotes increased interstitial lung edema in BPD. In future studies, we will perform experiments to inhibit miR-495 expression in the BPD model to assess interstitial lung edema and alveolar lumen fluid absorption. Thank you for your suggestion again.

References

[1] Bland RD, Carlton DP, Jain L. Lung fluid balance in neonatal development and lung diseases. In: Bancalari E, Polin RA, editors. The Newborn Lung: Neonatology Questions and Controversies. Elsevier (Singapore): Academic 2008; 128-149.

[2] Jain L, Eaton DC. Physiology of Fetal Lung Fluid Clearance and the Effect of Labor. Semin Perinatol 2006;30(1):34-43.

[3] Katz C, Bentur L, Elias N. Clinical implication of lung fluid balance in the perinatal period. J Perinatol 2011;31(4):230-5.

[4] Li T, Koshy S, Folkesson H G. Involvement of {alpha}ENaC and Nedd4-2 in the conversion from lung fluid secretion to fluid absorption at birth in the rat as assayed by RNA interference analysis. Am J

Physiol Lung Cell Mol Physiol 2007;293(4):L1069-L1078.
[5] Introvini P, Pagani G, Episcopi G, et al. Risk of bronchopulmonary dysplasia: the importance of diuresis. Pediatr Med Chir 1983;5(6):579-82.
[6] Clyman RI. Patent ductus arteriosus, its treatments, and the risks of pulmonary morbidity. Semin Perinatol 2018;42(4):235-242.

Comment 22: Figure 5 - Were you able to identify which cells were expressing NEDD4L and/or ENaC? The alveolar region is shown for NEDD4L, and the airway seems to show more ENaC. If that is the case, how will you associate both NEDD4L and ENaC? Please discuss.
Reply 22: NEDD4L and ENaC are expressed in both airway and alveolar epithelia. The alveoli were the subject of our study. To specific compare the changes in expression of NEDD4L and ENaC in the alveolar epithelial, we reselected the images. We have changed the images in Figure 5 as advised (See Page 21, Line 454-459).
Changes in the text: Figure 5.

Comment 23: Please make sure that you follow the correct gene and protein nomenclatures. Eg. Mouse proteins are capital and straight, mouse genes start with capital and italicized, etc.
Reply 23: We have modified our text as advised (See Page 11, Line 221-223; Page 21, Line 446-449).
Changes in the text: The RT-qPCR analyses also showed a significant decrease in *Nedd4l* expression and an increase in *ENaC* expression in the LPS-treated lungs at P3 (Figure 4A).
The expression of miR-495and *ENaC* was significantly increased in rat lungs of the LPS-treated groups versus the control group at P3. Decreased expression of *Nedd4l* in LPS-treated rat lungs was also detected.

Comment 24: Many experiments have n=3 numbers Since the experiments are on rat pups, you should get a good number of pups for each group. Please include data from at least 6 animals wherever possible.
Reply 24: Thank you for your advice. We included 6 animals per group in the RT-PCR experiment. In the western blot experiment, each protein sample was extracted from the lung tissues of three different rat pups, so there were 9 animals in each group. For morphological experiments, we included three animals in each group.

Comment 25: I would suggest doing at least some experiments to show that the inhibiting MiR-495 is protecting the lung morphology and rescuing the NEDD4L/ENaC expression. This would help suggest miR-495 to be a potential therapeutic target. Or include this in the future perspectives in the discussion part.
Reply 25: Thank you for your suggestion. We concluded that the aberrant overexpression of miR-495 might be involved in BPD by targeting NEDD4L/ ENaC. However, the current findings could not demonstrate that miR-495 overexpression causes more severe BPD injury through the NEDD4L/ENaC pathway. More experiments on inhibiting miR-495 overexpression *in vivo* need to be performed to prove the miR-495 mediated mechanism in BPD. We have added this in the perspectives in the discussion part as advised (See Page 14, Line 299-301).
Changes in the text: Further experiments on inhibiting miR-495 overexpression *in vivo* need

to be performed to prove the miR-495 mediated mechanism in BPD.

Comment 26: Lines 219-220'…which resulted in the overexpression of ENaC thus might increase the absorption of lung fluid…' needs to be changed as it looks like that parameter is not measured.
Reply 26: We have modified the sentence as advised (See Page 11, Line 233-236).
Changes in the text: The overexpression of miR-495 inhibited the expression of NEDD4L, which resulted in the overexpression of ENaC. ENaC has been reported to be a key regulator of fluid balance in lung development (16-17).

Comment 27: Lines 220-221- Current study suggests a novel therapeutic target of BPD must be modified as the effect of inhibiting miR-495 on protecting lungs is studied here.
Reply 27: We have changed the sentence as advised (See Page 11, Line 236-237).
Changes in the text: The findings suggest that miR-495 might be a potential therapeutic target for BPD.

Comment 28: Line 226 – sentence needs to be reconstructed.
Reply 28: The sentence has been reconstructed as "Yang and his colleagues found that miR-495 expression underwent significantly dynamic changes in rat lung development" (See Page 11-12, Line 241-244).
Changes in the text: Yang and his colleagues found that miR-495 expression underwent significantly dynamic changes in rat lung development. The expression of miR-495 was upregulated from canalicular to saccular stages and downregulated from saccular to alveolar stages.

Comment 29: Line 232 – '……patients, which was opposite to the findings in normal rat lung development'. What does this mean. Please clarify.
Reply 29: We have changed the sentence as advised (See Page 12, Line 246-248).
Changes in the text: Then, we detected increased expression of miR-495 in peripheral blood of BPD infants by RT-qPCR, which was opposite to the findings in normal rat lung development.

Comment 30: Line 250- I think it is Consistent, and not consisted
Reply 30: We have corrected the word in our text (See Page 13, Line 265-268).
Changes in the text: Consistent with previous studies, the intra-amniotic injection of LPS to SD rats at E16.5 in our study resulted in less and simplified alveoli compared with the control group which could be provided as an ideal animal model with pathological characteristics of BPD.

Comment 31: Line 253 – animal model.
Reply 31: We have corrected the error in our text (See Page 13, Line 265-268).
Changes in the text: Consistent with previous studies, the intra-amniotic injection of LPS to SD rats at E16.5 in our study resulted in less and simplified alveoli compared with the control group which could be provided as an ideal animal model with pathological characteristics of BPD.

Comment 32: Line 256 – change ‘was regulated’ to is regulating.
Reply 32: We have changed “was regulated” to “was to regulate” in our text based on the advice of you and other reviewers (See Page 13, Line 270-271).
Changes in the text: As mentioned above, the main function of NEDD4L, which was targeted by miR-495, was to regulate the expression of ENaC.

Comment 33: Line 282 – change ‘regulated’ to ‘regulate’
Reply 33: We have corrected the word in our text (See Page 14, Line 297-299).
Changes in the text: What’s more, our findings suggested that miR-495 targeted NEDD4L/ENaC pathway and might regulate the balance of lung fluid in BPD, but the molecular mechanism still needs further studies *in vitro* and *vivo*.

Comment 34: Lines 281-284 – Again says the involvement of MiR-459 in fluid balance, which is not studied. May be modified.
Reply 34: We have modified the sentence as advised (See Page 14, Line 297-299).
Changes in the text: What’s more, our findings suggested that miR-495 targeted NEDD4L/ENaC pathway and might regulate the balance of lung fluid in BPD, but the molecular mechanism still needs further studies *in vitro* and *vivo*.

Comment 35: Line 287 –‘….resulting in overabsorption of lung fluid’ needs to be changed if there is no supporting evidence from this current study.
Reply 35: We have modified the sentence as advised (See Page 14, Line 303-306).
Changes in the text: Moreover, miR-495 activated NEDD4L/ENaC pathway by inhibiting the expression of NEDD4L resulting in overexpression of ENaC, which has been reported to be important in the clearance of lung fluid.

Comment 36: Line 288-289 – sentence needs to be reconstructed. The term 'hypothesized’ needs to be changed and ‘could provide a new and promising therapeutic target for BPD treatment’ cannot be written as no inhibitor studies are done.
Reply 36: We have modified the sentence to “Our findings provide new avenues for exploring the pathogenesis and treatment in BPD” as advised (See Page 14, Line 306-307).
Changes in the text: Our findings provide a new avenue for exploring the pathogenesis and treatment in BPD.