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Research Paper

Epigenetic response to hyperoxia in the neonatal lung is sexually dimorphic

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ABSTRACT

Sex as a biological variable plays a critical role both during lung development and in modulating postnatal hyperoxic lung injury and repair. The molecular mechanisms behind these sex-specific differences need to be elucidated. Our objective was to determine if the neonatal lung epigenomic landscape reconfiguration has profound effects on gene expression and could underlie sex-biased differences in protection from or susceptibility to diseases. Neonatal male and female mice (C57BL/6) were exposed to hyperoxia (95% FiO, PND 1–5: saccular stage) or room air and euthanized on PND 7 and 21. Pulmonary gene expression was studied using RNA-seq on Illumina HiSeq 2500 platform and quantified. Epigenomic landscape was assessed using Chromatin Immunoprecipitation (ChIP-Seq) of the H3K27ac histone modification mark, associated with active genes, enhancers, and super-enhancers. These data were then integrated, pathways identified and validated. Sex-biased epigenetic modulation of gene expression leads to differential regulation of biological processes in the developing lung at baseline and after exposure to hyperoxia. The female lung exhibits a more robust epigenomic response for the H3K27ac mark in response to hyperoxia. Epigenomic changes distribute over genomic and epigenomic domains in a sex-specific manner. The differential epigenomic responses also enrich for key transcription regulators crucial for lung development. In addition, by utilizing H3K27ac as the target epigenomic change we were also able to identify new epigenomic reprogramming at super-enhancers. Finally, we report for the first time that the upregulation of *p21 (Cdkn1a)* in the injured neonatal lung could be mediated through gain of H3K27ac. These data demonstrate that modulation of transcription via epigenomic landscape alterations may contribute to the sex-specific differences in preterm neonatal hyperoxic lung injury and repair.

1. Introduction

The male and female neonatal lungs show differences in lung maturation during development. While other neonatal morbidities associated with preterm birth are on the decline, the incidence of bronchopulmonary dysplasia (BPD) remains high in the extremely premature population, as high as 40–50% [1]. BPD is characterized by impaired alveolarization and aberrant lung vascular development [2]. Increased oxidative stress through exposure to supraphysiological concentrations of oxygen (hyperoxia) contributes to the pathogenesis of this disease. Premature male neonates have a higher risk of developing this disease, and we and others have shown this to be true in animal models of BPD as well [3,4]. The mechanisms underlying sexually dimorphic pathophysiology in the development of this disease still need to be elucidated.

We have previously reported sex-specific differences in the whole lung transcriptome, after hyperoxia exposure during the saccular phase of lung development (PND1-5) and that this difference becomes more striking during recovery at the late alveolar phase (at PND21) [5]. Epigenomic landscape reconfiguration, with profound effects on gene expression, is an important regulatory mechanism shown to play a role in diverse biological processes. The interplay between epigenomic

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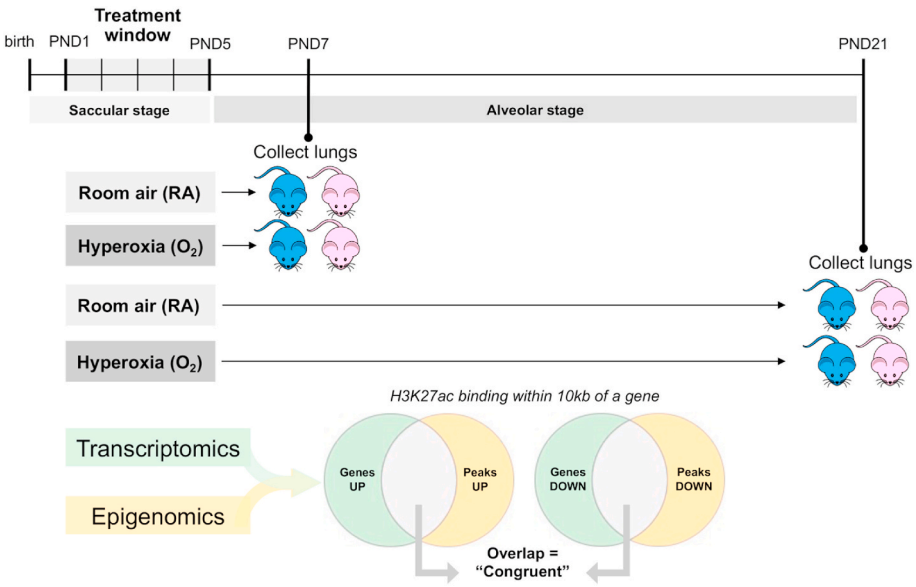

Fig. 1. Integrated epigenomic and transcriptomic strategy. We generated ChIP-Seq profiles for H3K27ac histone modification, and determined significant changes using DiffReps. We used our now publicly available RNA-Seq data (GSE97804) from our lab and generated gene signatures using DESeq2. We integrated the transcriptomic and the epigenomic data by identifying coordinated epigenomic and transcriptomic changes.

changes and transcriptomic changes is complex. DNA methylation, transcription factors, microRNAs, and numerous histone modifications, including H3K27ac, can all contribute towards an intended gene expression change, with the results highly context-specific. H3K27ac is an epigenetic modification involving acetylation of the 27th lysine residue of the histone protein H3. It is associated with the location of active and poised enhancers and activation of transcription. The sex-specific differences in the epigenomic landscape, due to changes in the H3K27ac mark in the neonatal lung at baseline and after exposure to early hyperoxic injury, have not been previously studied. We decided to focus on the H3K27ac mark, as it enables the interrogation of super-enhancers, which are regions in the mammalian genome comprised of multiple enhancers with high levels of transcription factors and control genes that are critical for cell type specification [6]. The objective of this study was to perform an integrative epigenomic and transcriptomic analysis of sex-biased differences in a murine model of neonatal hyperoxic lung injury (Fig. 1). The overall hypothesis of this study was that there are sex-specific differences in the neonatal lung epigenome for the H3K27ac mark at baseline and upon exposure to hyperoxia, and that these alterations drive some of the key differences in the pulmonary transcriptome.

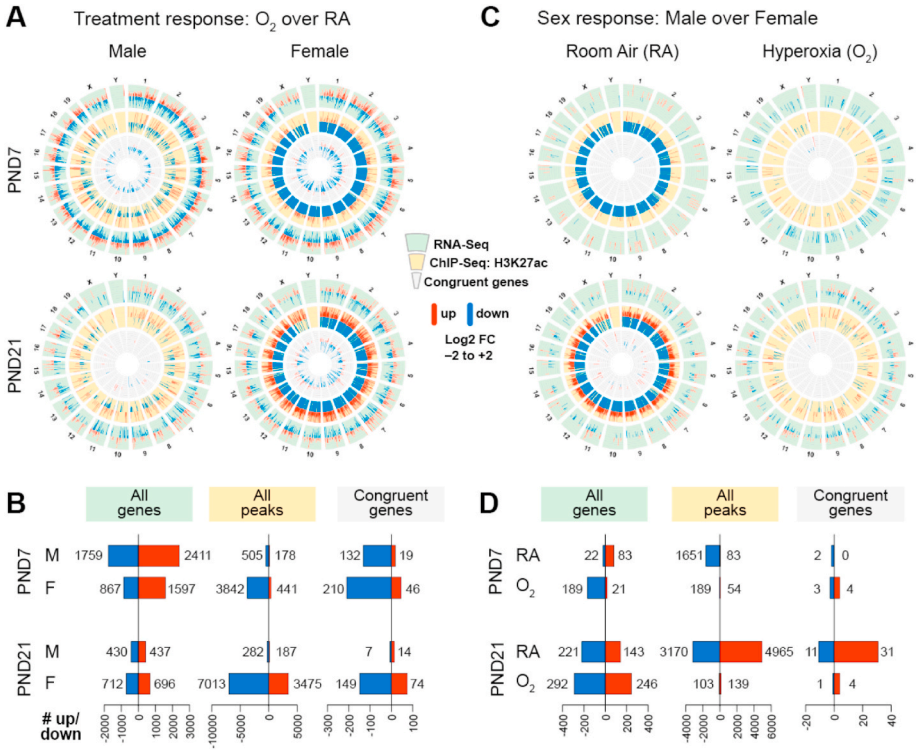

Fig. 2. Congruent epigenomic and transcriptomic responses show sex-specificity A. Response to hyperoxia in neonatal male and female lung on PND7 and PND21 is depicted using Circos plots, including all differential genes (outermost zone, green), all differential H3K27Ac peaks (middle zone, yellow), and genes with congruent epigenomic/transcriptomic response (innermost zone, grey). The increase in ChIP-Seq and RNA-Seq signal is shown in red and the decrease in signal is depicted in blue, shown as log 2 fold changes. B. Summary of changes in response to hyperoxia within each sex at PND7 and PND21. C. Differences between male and female neonatal mouse lung RNA-Seq and H3K27Ac ChIP-seq data are depicted using Circos plots at baseline (normoxia) and upon exposure to hyperoxia at PND7 and PND21. Data is represented similarly to that described in 2A. D. Summary of changes between male and female neonatal lung in normoxia or after exposure to hyperoxia at PND7 and PND21. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

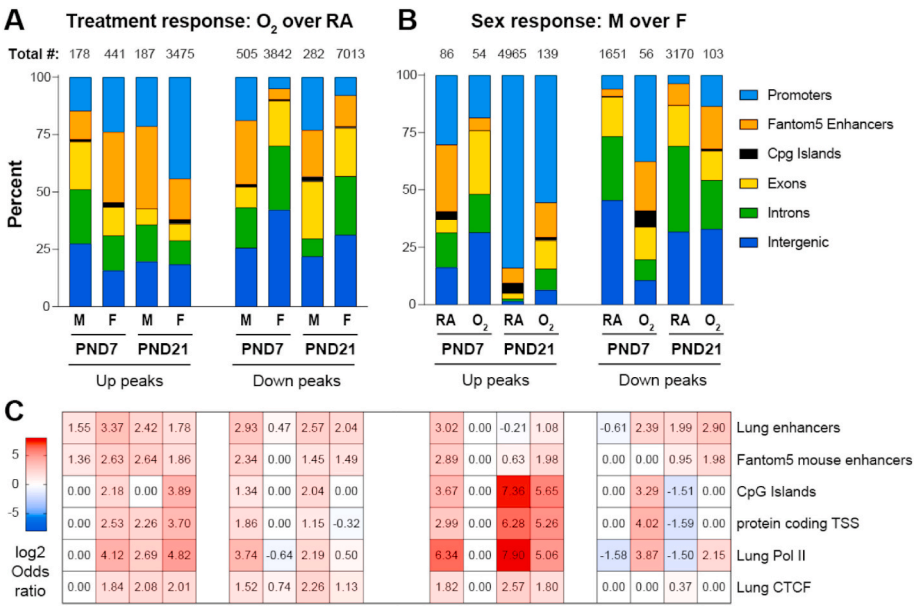

Fig. 3. Distribution of H3K27ac changes over known genomic and epigenomic domains. A In hyperoxia exposed male and female neonatal mice compared to sex-matched room air controls. Induced and suppressed peaks are shown separately. B. In room air and hyperoxia-exposed neonatal male mice compared to similar exposed female mice. Induced and suppressed peaks are shown separately. C. Odds-ratio enrichment of differential H3K27ac peaks with epigenomic domains for each of the comparisons specified above.

2. Methods

2.1. Mouse model of bronchopulmonary dysplasia

Care and handling of mice occurred according to an animal protocol approved by the Institutional Animal Care and Use Committee (IACUC) at Baylor College of Medicine. C57BL/6 mice (from Charles River Laboratories) were used for these experiments. The anogenital distance and pigmentation in the anogenital region method was used in determining the sex [7]. On PND7, we reconfirmed the sex of the mice with PCR analysis for the *Sry* gene. Mouse pups were exposed to hyperoxia (95% FiO_2), as described previously [3] within 12 h of birth for 5 days. Following euthanasia by approved methods, lung tissues were collected on PND7 and PND21 (after recovery in room air) as most of postnatal lung development in mice is completed by this age [8].

Lungs were subjected to RNA-Seq and ChIP-Seq analysis at these time-points. (Fig. 1). The supplemental data provides a detailed description of the RNA-Seq and ChIP-seq data acquisition and analysis.

2.2. Human studies

Deidentified lung protein and mRNA samples from BPD patients and age-matched controls were kindly provided by Dr. Gloria Pryhuber (University of Rochester Medical Center, Rochester), obtained under protocols approved by the IRB of the University of Rochester after obtaining informed consent. Results from these archived human lung BPD samples (with details of inclusion/exclusion criteria, sample collection/storage methodology) have been extensively reported [9–11]. The supplemental data provides detailed descriptions of Western blot and qPCR methodology.

2.3. Statistical analysis

Data is expressed as mean ± SD and analyzed by two-way ANOVA to test for both independent and interaction effects of sex and hyperoxia, followed by Bonferroni correction.

3. Results

3.1. Congruent epigenomic/transcriptomic responses show sex-specificity

To evaluate our observations in context of the inherent biological sex differences, we carried out two comparisons: first, the response to hyperoxia, defined as differences between hyperoxia and room air, in each sex, at each time point (Fig. 2A) and second, the differences between males and females, within each exposure group, at each time point (Fig. 2C).

Females showed significantly greater loss of H3K27ac peaks both at PND7 (3842 in female compared to 505 in male) and 21 (7013 in females compared to 282 in males). The gain in H3K27ac mark was also more pronounced in the female lung in response to hyperoxia compared to the male lung, but to a lesser extent. At PND7, the H3K27ac epigenomic response to hyperoxia in both sexes suppressed more peaks that it induced (Fig. 2B), whereas the corresponding transcriptional response was upregulated.

To focus on genes where H3K27ac epigenomic changes are more likely to have a functional effect, we considered the *congruent epigenomic and transcriptomic response*, including differentially expressed genes, with at least one differential H3K27ac site within 10 kb from the gene body and significantly altered in the same direction as the gene, and with no differential binding sites within 10 kb of the gene body altered in the opposite direction to the gene expression (Suppl Fig E1). At PND7, in male mice hyperoxia exposure induced 19 and suppressed 132 congruent genes, whereas in females it induced 46 and suppressed 210 congruent genes (Fig. 2B). In both sexes hyperoxia suppressed more congruent genes that it induced, in agreement with the standalone epigenomic response. At PND21, there was a higher ratio of decreased congruent genes in females compared to males (p-value<0.004). At PND7 under room air (Fig. 2D), there was a higher proportion of up-regulated genes in males compared to females in room air, and a higher proportion of down-regulated genes in hyperoxic conditions. At PND21, the difference in ratio between the number of increased and decreased genes between males and females was not significantly different between room air and hyperoxia (p-value<0.065). In room air, the males showed a striking number of decreased H3K27ac peaks.

At PND21, the absolute number of differential peaks detected in the room air group versus the hyperoxia group greatly differed but there were similar ratios of increased and decreased peaks in each group (p-value<0.257). A distribution of differential peaks sizes in hyperoxia response and between biological sexes is presented in Suppl Fig E2.

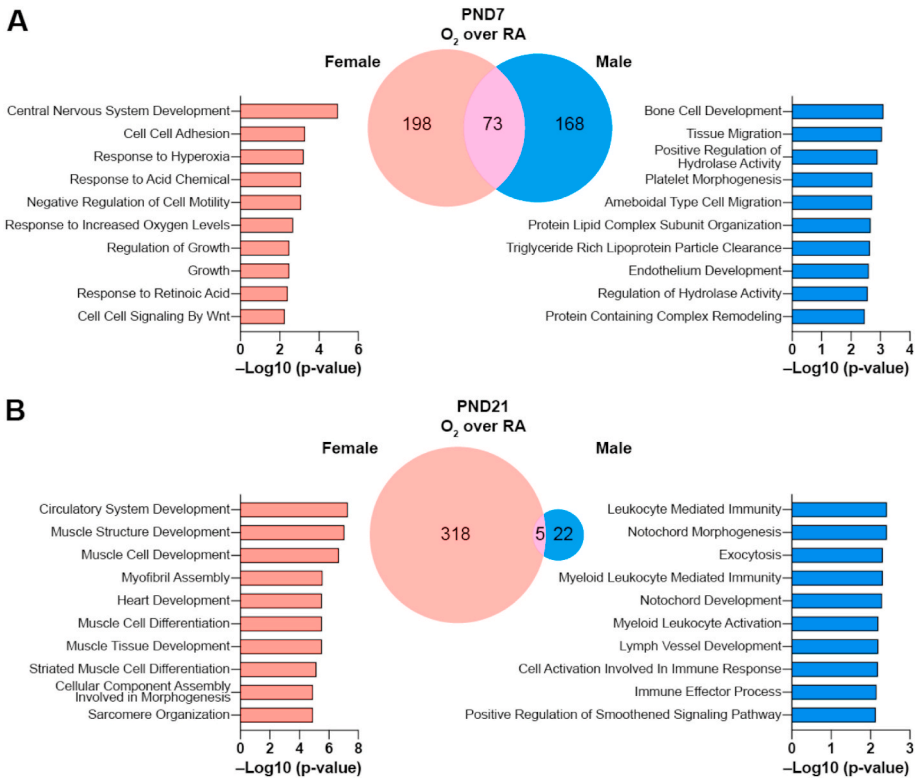

Fig. 4. Congruent genes show distinct modulations of biological pathways in male and female neonatal mice exposed to hyperoxia.

3.2. *Epigenomic changes distribute over genomic and epigenomic domains in a sex-specific manner*

We characterized the relative distribution of increased and decreased H3K27ac binding for the exposure response (Fig. 3A) and sex-specific response in normoxic and hyperoxic conditions (Fig. 3B). When comparing the *up versus down H3K27ac peaks in each sex in response to hyperoxia* (Fig. 3A), there is a higher overlap of down H3K27ac peaks at PND7 and up peaks at PND21 at enhancers in male mice. In females, we noticed higher overlap of up peaks with promoters and enhancers at PND7 and PND21. With regards to *male versus female distribution of H3K27ac peaks in response to hyperoxia* (Fig. 3A), females showed a higher overlap of up peaks at enhancers (PND7) and at promoters (PND21), while males showed a higher overlap of down peaks at promoters and enhancers at PND7 and PND21. Overall, the odds-ratio overlap of H3K27ac differential peaks with both genomic and epigenomic elements shows sex specificity (Fig. 3C).

3.3. *Congruent epigenomic/transcriptomic responses enrich for key pathways*

At PND7, upon hyperoxia exposure, congruent genes enriched for 198 pathways unique to females and 168 pathways unique to males; 73 pathways were common to both sexes (Fig. 4A). Response to hyperoxia and increased oxygen levels were uniquely enriched in females due to altered expression of genes including: *Cyp1a1, Cdkn1a (p21), Col1a1, Foxo1,* and *Pdgfrb*. The expression of *p21* and *Col1a1* was increased and associated with H3K27ac gain, while expression of *Foxo1, Pdgfrb and Cyp1a1* was decreased and associated with H3K27Ac loss. The role of *Cyp1a1* and *p21* in hyperoxic lung injury has been reported in previous publications [3,12]. *Col1a1* is upregulated in hyperoxia exposed fibroblasts [13]. *Foxo1* was downregulated in small pulmonary arteries in pulmonary hypertension and *Foxo1* reconstitution reversed vascular remodeling [14]. PDGFR-β contributed to hypoalveolarization in BPD and expression was decreased in mesenchymal stem cells from infants developing BPD [15].

Biological processes enriched in the congruent genes of hyperoxia-exposed male and female neonatal mice were identified using over-representation analysis (ORA) as implemented by the Molecular Signature Database (MSigDB). The Normalized Enrichment Score (NES) is reported for select enriched pathways on the X-axis (fdr-adjusted Q-value<0.25). A: Unique pathways involving the congruent genes in male and female neonatal mice exposed to hyperoxia on PND7. B: Top 10 unique pathways involving the congruent genes in male and female neonatal mice exposed to hyperoxia on PND21.

Pathways unique to males at PND7 included endothelium development, bone cell development, and tissue migration. The endothelium development pathway included the congruent downregulated genes *S1pr1*, *Sox17, Bmpr2 and Cdh5*, associated with H3K27ac loss. Endothelial sphingosine-1-phosphate receptor 1 (*S1pr1*) amplifies VEGFR2-mediated angiogenic signaling [16]. *Sox17* is required for normal lung vascular morphogenesis [17], while *Bmpr2* depletion is associated with pulmonary fibrosis and pulmonary hypertension [18].

At PND21, congruent genes enriched for 318 pathways unique to females and 22 pathways unique to males; only 5 pathways were common to both sexes, compared to 73 at PND7 (Fig. 4B). Muscle structure and circulatory system development were unique to females, and included *Slit2*, a gene upregulated and associated with gain of three H3K27ac peaks. SLIT2 secretion by fibroblasts prevented fibrosis [19] and, in postnatal murine retina, SLIT2 promoted angiogenesis [20]. Interestingly, at PND21 pathways unique to males included immune-related processes such as myeloid leukocyte activation and immune effector process. Integrin Subunit Alpha X (*Itgax*), or *Cd11c,* was an upregulated gene accompanied by H3K27ac gain. It plays a role in adherence of monocytes to VCAM-1 [21]. CD11c is a marker of alveolar and interstitial macrophages and dendritic cells in the lung [22]. We previously reported that male lungs showed greater macrophage infiltration compared to female, in association with a greater lung vascular development impairment [3].

Since the epigenomic response was mainly manifested as loss of

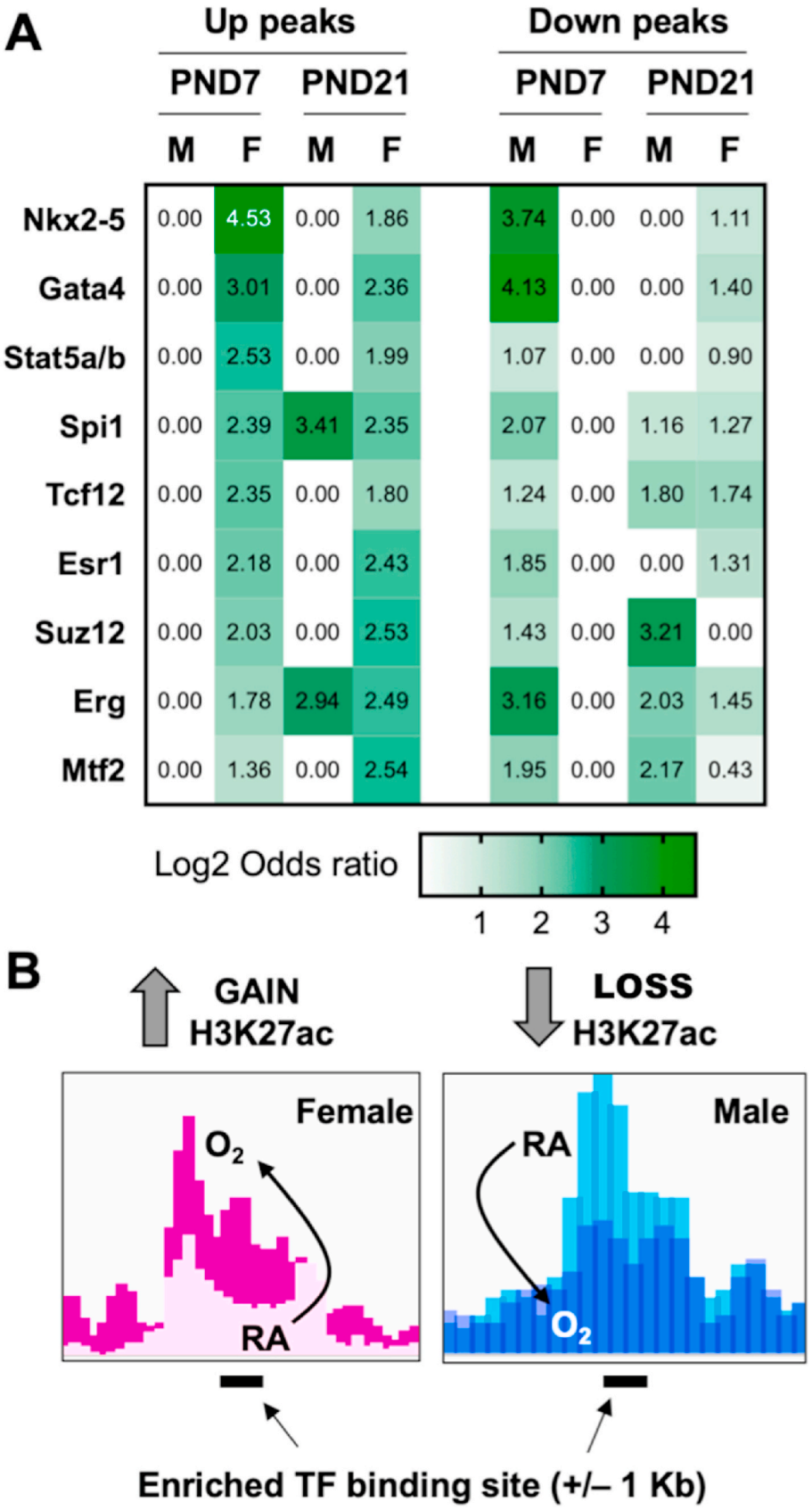

Fig. 5. Epigenomic response to hyperoxia enriches for transcription factors in a sex-specific manner. A. The epigenomic response to hyperoxia shows enrichment in up-regulated genes in females and down-regulated genes in males for related transcription regulators such as ESR1, GATA4, NKX2-5, and ERG. B. Graphical model of preferential sex-specific enrichment for transcription factors in increased or decreased H3K27ac peaks in response to exposure to hyperoxia.

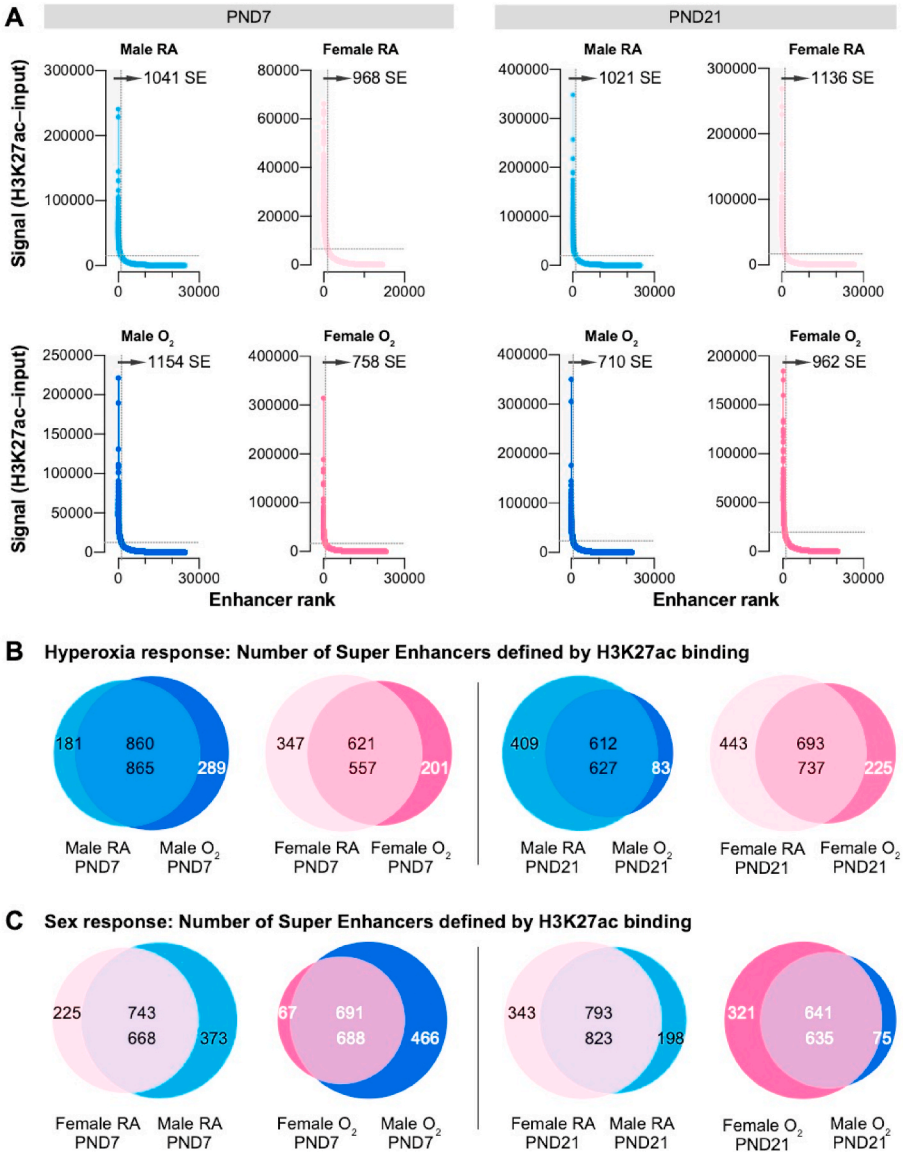

Fig. 6. Super-enhancers in the neonatal mouse lung show distinct sex-specific profiles and a robust hyperoxia response. A. Selection of Super-enhancers using the ROSE algorithm. All candidate enhancer clusters were ranked by slope; final super-enhancers numbers are indicated for each experiment. B. At PND7 hyperoxia exposure leads to an increase in the super-enhancer number in males and a corresponding decrease in females. At PND21, hyperoxia exposure leads to a robust reduction in the super-enhancer number for both sexes. C. At PND7 males have a higher number of super-enhancers than females in both Room Air males in both Room Air and Hyperoxia conditions.

H3K27ac peaks, we correlated the expression of decreased congruent genes with publicly available neonatal lung single-cell expression database (LungMAP) [23] in Suppl Fig E3. Interestingly, many of these genes were involved in vasculature development and enriched in vascular endothelial cells (*Tmem 100, Ptprb, Cdh5, Sox17, Aplnr, ppp1r16b, and Ahr*).

3.4. Epigenomic responses enrich for key transcription regulators

We next identified key transcription factors (TFs) that showed distinct enrichment patterns between hyperoxia affected peaks in males and females. We determined TFs gained in vicinity of hyperoxia increased H3K27ac peaks in females and decreased H3K27ac peaks in males at PND7 (Fig. 5). These include TFs such as NKX2-5 (NK2 Homeobox 5), GATA4 (Gata binding protein 4), STAT5A (Signal Transducer and Activator of Transcription 5A), ESR1 (Estrogen receptor 1), SUZ12 (SUZ12 polycomb repressive complex 2 subunit, ERG (ETS Transcription Factor ERG) and MTF2 (Metal Response Element Binding Transcription Factor 2). NKX2-5 is involved in regulation of myofibroblast differentiation and is decreased in the *in vivo* bleomycin model of lung injury and fibrosis [24]. ERG target genes were associated with a H3K27ac gain in females at both PND7 and 21. Conversely, it was associated with a loss of H3K27ac peaks at PND7 in males. ERG is endothelial-specific and essential for endothelial cell homeostasis, and drives expression of lineage-specific genes [25]. ERG expression in pulmonary endothelium was significantly reduced in human pulmonary hypertension and in chronically hypoxic mice [26]. The complete list of transcription factors (TFs) associated with the epigenomic response and changes in each exposure and control group are shown in Suppl Fig E4.

3.5. Hyperoxia leads to sex-specific super-enhancer landscape changes

Super-enhancers (SEs) are discrete genomic regions gaining recognition both as a distinct mode of transcription regulation [17] and as potential therapeutic targets [27]. We assessed the SE landscape change in each exposure group (Fig. 6A). This approach yielded 758–1154 SEs per experimental group at PND7 and 710–1136 SEs at PND21, in line with the number of SEs reported in prior studies [25].

At PND7, hyperoxia exposure in males led to a loss of 181 SEs (17.4% of total SEs) and a gain of 289 SEs, for a net 108 SEs gain. However, hyperoxia exposure in females yielded the loss of 347 SEs (35.8% of total SEs) and a gain of only 201 SEs, for a net loss of 146 SEs. At P21, we observed a net loss of SEs for both males and females after hyperoxic exposure (Fig. 6B). At PND7 males showed a higher super-enhancer

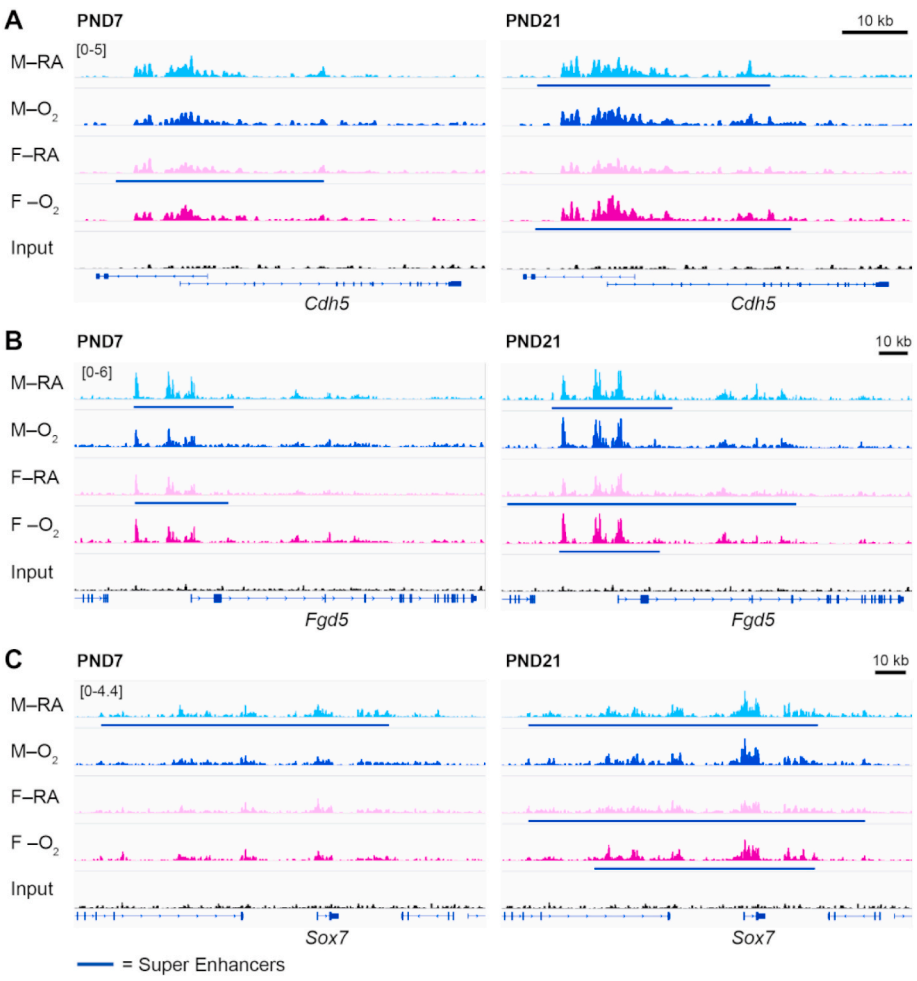

Fig. 7. Individual super-enhancers associated with the lung response to hyperoxia. A. A super-enhancer at the Cdh5 locus was determined in female lungs in normoxic conditions at PND7 and after exposure to hyperoxia at PND21. B. A super-enhancer at the Fgd5 locus was lost both in males and females at PND7 upon exposure to hyperoxia and was associated with a decrease in gene expression in both males and females C. A super-enhancer at the Sox7 locus was lost in the male lung at both PND7 and PND21 upon exposure to hyperoxia.

number in both room air and hyperoxia, Interestingly, at PND21 this shifted to females (Fig. 6C). Thus, there was a notable difference in the SE landscape between the male and female lung at different stages of lung development at PND7 (early alveolar) to PND21 (late alveolar). The genomic locations of SEs in each of the experimental groups are provided in Supplemental Table 1, whereas genes within 10 Kb of SEs are provided in Supplemental Table 2.

As an example of SE lost in females at PND7 upon hyperoxia exposure and gained back at PND21, we show the *Cdh5 (VE-Cadherin)* locus (Fig. 7A). The Cdh5 SE locus has been previously reported in human umbilical vein endothelial cells [25]. *Cdh5* is endothelial-specific and plays an important role in vascular morphogenesis and growth. Another interesting SE was identified at the *Fgd5* (FYVE, RhoGEF and PH Domain Containing 5) locus [28]. *Fgd5* is expressed in cultured human vascular endothelial cells and mediates many VEGF-mediated actions including endothelial cell proliferation and capillary network formation. One of the mechanisms was through inhibition of proteasome-dependent VEGFR2 degradation [29]. H3K72ac peaks defining this super-enhancer locus were lost both in males and females at P7 upon hyperoxia exposure, associated with decreased gene expression in both sexes (Fig. 7B). The *Sox7* SE locus was lost in male lungs at both PND7 and PND21 upon hyperoxia exposure (Fig. 7C). SOX7 was one of the earliest transcription factors found to be increased in HUVECS subjected to hypoxia and loss of SOX7 decreased tube formation indicating its role in recovery after vascular injury. Furthermore, *SOX7* mediated the shift to glycolytic metabolism by HIF-1α and HIF-2α mediated angiogenesis in hypoxia-exposed endothelial cells [30]. *Cdh5* was identified as a downstream *Sox7* transcriptional target in hemogenic endothelium [31].

3.6. Upregulation of p21 in neonatal hyperoxic lung injury is associated with H3K27Ac gain

Among congruent genes common to males and females at P7 in response to hyperoxia exposure was *Cdkn1a,* or *p21*. We validated p21 expression in an independent cohort of animals to confirm our findings. As shown in Fig. 8A, H3K27ac signal is robustly increased at the *p21* promoter. Results of ChIP-qPCR validation for *p21* in an independent mouse cohort are shown in Fig. 8B. There was enrichment of the H3K27ac mark at the *p21* locus upon exposure to hyperoxia at PND7, when both male and female samples were combined. Protein and mRNA p21 expression was increased *in vivo* in both male and female neonatal lungs at PND7 immediately after hyperoxia exposure (Fig. 8C). Furthermore, p21 expression was also increased in human BPD lungs compared to controls (Fig. 8D). Thus, we showed that altered p21 expression could be influenced by altered H3K27ac-mediated transcriptional activation.

4. Discussion

The integrative analysis of sex-specific epigenomic and transcriptomic responses in hyperoxia-exposed neonatal lung is, to our knowledge, the first of its kind. Dysregulation of transcription via epigenomic landscape alterations may contribute to sex-specific differences in hyperoxia-induced inhibition of lung development and to diseases, such as BPD. Notably, more gene changes associated with epigenomic

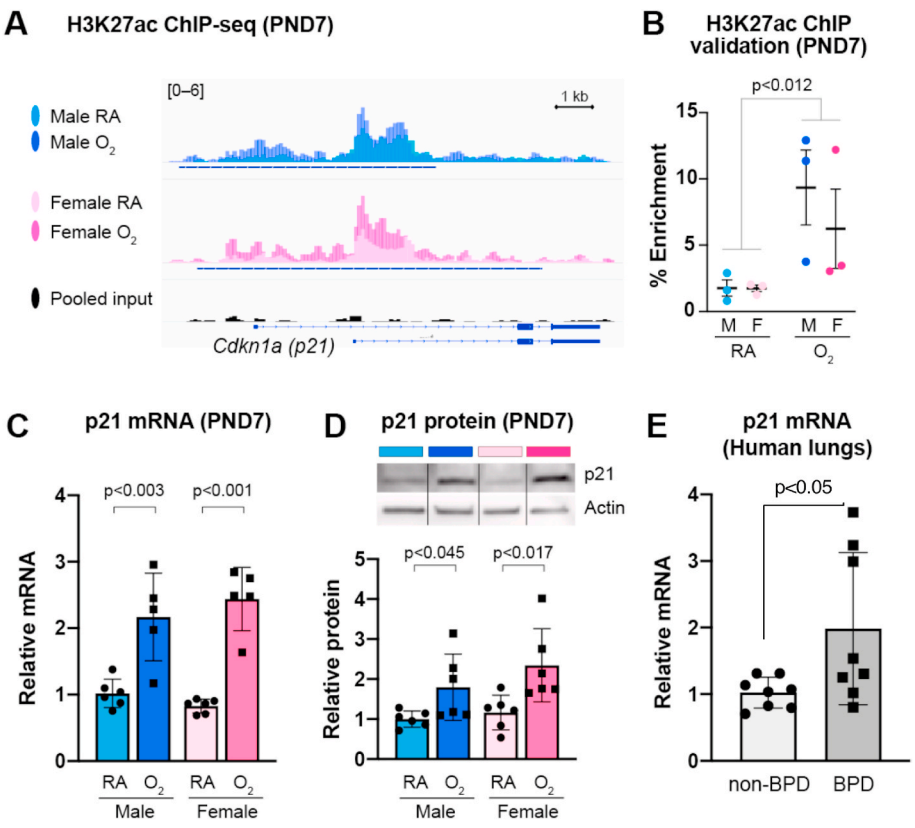

Fig. 8. CDKN1A (p21) activation in neonatal lung upon hyperoxia exposure is associated with a gain of H3K27Ac signal and expression is increased *in vivo* in the murine model and in human BPD lung samples: A. Hyperoxia-exposed male and female neonatal lung (95% FiO_2, PND1-5) shows a gain of H3K27ac peaks at the p21 locus at PND7. B. An independent ChIP-qPCR experiment showed enrichment of H3K27ac signal at the p21 locus in male and female neonatal lungs (taken together; n = 6/group). C. *p21* mRNA and protein expression (D) was increased in male and female mice *in vivo* in the lung at PND7 after exposure to hyperoxia (n = 5–6/group). E. *p21* mRNA expression was increased in human BPD lung samples compared to age-matched controls (n = 8/group). Data is shown as mean ± SD. Statistically significant differences between the groups is represented by bars.

changes included down-regulated genes and loss of H3K27ac. We identified enriched pathways unique to each sex, based on the overlap between the transcriptomic and epigenomic signature (congruent genes). By utilizing H3K27ac as a target epigenomic mark we identified epigenomic reprogramming at super-enhancers. Finally, we report for the first time that the upregulation of *p21 (Cdkn1a)* in neonatal hyperoxic lung injury could be mediated through H3K27ac gain in the acute phase of the murine model and we also showed increased p21 expression in human BPD lung samples.

The interplay between epigenomic and transcriptomic changes is complex. Previous studies examining the role of epigenetic modifications in neonatal hyperoxic lung injury have focused mainly on DNA methylation [32–34]. Cuna et al. studied alterations in gene expression and DNA methylation in normal murine and human BPD and control lung samples. In mice, they found 95 genes with inverse correlation between gene expression (microarray) and methylation (MeDIP-Seq) [35].

Among histone modifications, the role of EZH2-mediated H3K27me3 was studied at the RUNX3 promoter under hyperoxia [36]. Microarray profiling of umbilical cord tissues from 54 premature neonates in a 2007 study revealed histone acetyltransferase binding activity and chromatin remodeling pathways as being significantly differentially regulated in babies that developed BPD, and a trend towards decreased HDAC1 expression in the BPD population [37]. HDAC1 and HDAC2 expression in alveolar and airway epithelium was decreased in FVB neonatal mice exposed to postnatal hyperoxia [38]. Decreased HDAC activity and increased HAT activity has been reported in many other chronic debilitating diseases including asthma and COPD [39]. Interestingly, corticosteroids, which are used both antenatally and postnatally in premature neonates, exert part of their anti-inflammatory effect in lung diseases such as asthma through HAT activity inhibition and by HDAC2 recruitment to the activated inflammatory gene complex [40]. Our study identified a role for the H3K27ac histone modification in a mouse model of hyperoxic lung injury and highlighted sex-specific differences. Neonatal mice are at the saccular stage of lung development during this period, which is equivalent to 26–36 weeks in human neonates. This leads to a pronounced arrest of lung development and is similar to the disease phenotype seen in human neonates with BPD. The female lung exhibits a more robust epigenomic response for the H3K27ac mark in response to hyperoxia and this response becomes more stark at PND21 during the recovery and repair phase. Changes in the H3K27ac epigenomic mark accounted only partially for the observed transcriptional response or these epigenetic changes due to alterations in the H3K27ac mark may not be associated with a change in gene expression and manifest as silent reprogramming.

We highlighted sex-specific pathways based on a congruent transcriptomic and epigenomic response. Constituent genes of these enriched pathways provide candidates for further elucidation of sex-specific mechanisms in neonatal lung injury and repair, but also highlight the epigenomic-mediated mechanism of transcriptional regulation in hyperoxic lung injury. Similar to our previous report [5], where transcriptomic response between males and females became more divergent at PND21, pathways enriched in congruent genes in this study showed reduced overlap between males and females at PND21 compared to PND7. Enrichment of key transcription factors (TFs), such as NKX2-5 and ERG, to these regulated genes could also explain the activation/suppression of the TF target genes with gain/loss of H3K27ac peaks, requiring further elucidation. Super-enhancer landscape alterations associated with neonatal hyperoxic lung injury have not been reported previously. Using the H3K27ac epigenomic mark we identified super-enhancers in our murine neonatal lung injury model. The super-enhancer landscape was notably different between males and females both at baseline (normoxia) and upon hyperoxia exposure.

Lung p21 expression was increased upon hyperoxia exposure in murine and non-human primate models *in vivo,* and pulmonary epithelial cells *in vitro* [38,41–43]. P21 upregulation was thought to be protective. However, prolonged growth arrest in the developing lung could lead to impairment of lung growth and maturation. P21 is a marker of

cellular senescence, contributing to cellular aging in many chronic lung diseases [44,45]. Initially thought to be dependent on p53 [41], it was later shown that p21 could be induced in hyperoxia independently of p53 [46]. Interestingly, in the absence of p21, mortality and alveolar cell death was increased, and repair of the hyperoxia-injured lung was hampered [12,46,47]. Moderate hyperoxia (40% FiO_2) upregulated p53 and p21 in human fetal lung fibroblasts and airway smooth muscle cells [48,49]. In this study, we show that the p21 upregulation in the acute phase after hyperoxia exposure could be mediated through H3K27ac gain.

We realize that many other epigenetic modifications could underlie the sex-specific differences in the lung transcriptome and worthy of further studies. We decided to focus on the H3K27ac mark, as it enables the interrogation of super-enhancers, which are regions in the mammalian genome comprised of multiple enhancers with high levels of transcription factors and control genes that are critical for cell type specification [6]. As such, this is the first report of sex-specific epigenomic reprogramming at super-enhancers in the developing lung exposed to hyperoxia. To prove that H3K27ac epigenomic changes in the mouse lung drive sex-specific gene expression changes, mechanistic studies that target these genes and their regulation by the specific histone acetylation using *in vivo* models would be required. Whole mouse lungs were used in our study, and thus the signal is an aggregate from multiple cell types and compartments making cell-type specific changes difficult to predict from this study. Nevertheless, our study has several important strengths. This is the first study looking at the differences in histone acetylation (specifically H3K27ac) in the developing mouse lung during early and late alveologenesis both at baseline and after hyperoxia exposure during the saccular stage of lung development. Integration with transcriptomic data led to the identification of novel candidate genes for future research and provided insight into epigenetic mechanisms regulating observed gene expression changes. The use of human BPD and preterm and term control lung tissue samples lends human relevance of these findings in the mouse model. These data highlight that epigenomic landscape alterations may contribute to the sex-specific differences in preterm neonatal lung injury and repair.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2020.101718.

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