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# The Renin Cell Baroreceptor, A Nuclear Mechanotransducer Central for Homeostasis

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## Abstract

**Rationale:** Renin-expressing cells are myoendocrine cells crucial for survival. They have been postulated to possess a pressure-sensing mechanism, a baroreceptor, that can detect slight changes in blood pressure and respond with precise and synchronized amounts of renin synthesized and released to the circulation to maintain blood pressure and fluid-electrolyte balance. The location and nature of this puzzling pressure-sensing structure have remained unknown since it was originally suggested over sixty years ago.

**Objective:** To elucidate the location and structure of the renin cell baroreceptor.

**Methods and Results:** We used a variety of genetically modified mice whereby renin cells were exposed *in vivo* to either low or high arterial pressure. In addition, we applied direct mechanical stimuli, i.e., pneumatic pressure or stretch, directly to renin cells cultured under different conditions and substrata. Changes in perfusion pressure and/or direct mechanical stimuli induced significant changes in renin gene expression and the phenotype of renin cells. Importantly, the experiments show that the pressure-sensing mechanism (the baroreceptor) resides in the renin cells; it requires initial extracellular sensing by integrin β1 at the renin cell membrane and is transduced to the nuclear membrane and chromatin by lamin A/C.

**Conclusions:** These studies show that the enigmatic baroreceptor is a nuclear mechanotransducer that resides in the renin cells *per se* and is responsible for the sensing and transmission of extracellular physical forces directly to the chromatin of renin cells via lamin A/C to regulate renin gene expression, renin bioavailability, and homeostasis.

### Keywords

Renin; blood pressure; perfusion; gene expression; mechanotransduction

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DISCLOSURES

None.

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**Subject Terms:**

ACE/Angiotensin Receptors/Renin Angiotensin System; Nephrology and Kidney

## INTRODUCTION

Renin-expressing cells are essential for survival, perfected throughout evolution to maintain blood pressure (BP) and fluid-electrolyte homeostasis[1]. In adult mammals, renin cells are strategically located at the juxtaglomerular (JG) tip of the afferent arterioles as they enter the glomeruli[2]. Renin cells are sensors endowed with the ability to detect and respond to minute changes in BP and the composition and volume of the extracellular fluid. In response to a decrease in perfusion pressure, renin cells synthesize and release renin to the circulation resulting in the restoration of BP[1]. An increase in pressure, on the other hand, results in decreased renin release, thus preventing the development of hypertension[1]. How renin cells sense and respond to changes in pressure is unknown. In 1957 Tobian and coworkers[3] postulated the existence of a pressure sensing-mechanism (denominated the baroreceptor) in the control of renin release. However, the nature and location of this baroreceptor and how it controls renin expression are unclear.

Using a variety of novel *in vivo* and *in vitro* approaches, we tested the hypothesis that this pressure-sensing mechanism, the enigmatic baroreceptor, is a nuclear mechanotransducer that resides in the renin cells and is responsible for the sensing and transmission of extracellular physical forces directly to the chromatin of renin cells to regulate renin gene expression, renin bioavailability, and renin cell phenotype.

## METHODS

Detailed methods are available in the Online Supplement. Please, see also the Major Resources Table in the Supplemental Materials.

### Data Availability.

All supporting data are available within the article and its supplementary files. The RNA-seq and ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) data sets generated in this study can be accessed at the GEO public repository using the accession number GSE157699 and GSE167522, respectively. R codes are available on Zenodo at https://doi.org/10.5281/zenodo.4672146. Additional information will be available upon request to the corresponding authors.

## RESULTS

### Perfusion pressure controls the expression of the renin gene.

To investigate whether changes in perfusion pressure affect the expression of the renin gene, we implemented a model of aortic coarctation (AoCo) between the roots of the right and left renal arteries (Figure 1A). In this model, the right and left kidneys are subjected to high and low perfusion pressure, respectively, allowing the study of the kidneys from the same mouse

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simultaneously, eliminating effects due to genetic background and individual differences. We confirmed this surgical model's validity by simultaneously measuring the intraarterial BP above (carotid artery) and below (femoral artery) the constriction three days after the AoCo. BP in the carotid arteries was significantly higher than in the femoral arteries in mice subjected to the AoCo, (Figure 1B). Three days after the AoCo, we extracted RNA from the renal cortices. Using quantitative reverse transcription PCR (qRT-PCR) for *Ren1*, there was no difference between the right and left renal cortices of mice subjected to sham surgeries. However, the renal cortices from mice subjected to the AoCo showed significant differences in *Ren1* mRNA in both female and male mice: lower expression in the right kidneys (exposed to higher perfusion pressure) and higher expression in the left kidneys (exposed to lower perfusion pressure) (Figure 1C, D). *Ren1* expression in the right kidneys and left kidneys with AoCo was significantly lower in the right and higher in the left than in mice subjected to sham surgeries (Online Figure I). By *in situ* hybridization (ISH), the intensity and extension of the *Ren1* mRNA signals at the JG area were lower in the right and higher in the left kidneys of the mice subjected to AoCo, compared to the kidneys from mice with sham surgeries (Figure 1E). Overall, our *in vivo* model to study the effects of changes on renal perfusion pressure showed that the *Ren1* expression in renin cells was decreased by high perfusion pressure and increased by low perfusion pressure, respectively.

## Renin cells switch phenotype in response to changes in perfusion pressure.

To gain insight into the mechanisms involved in the transformation of renin cells in response to changes in perfusion pressure, we performed a transcriptome analysis of renin cells in the kidneys with AoCo (Figure 2A). We used the *Ren1$^{c}$-YFP* mice that express a yellow fluorescent protein (YFP) under the control of 4.9 kb of the 5' regulatory region of the renin gene[4] and isolated YFP-positive cells using fluorescence-activated cell sorting[5]. With AoCo, the percentage of YFP-positive cells captured from the left renal cortices was significantly higher than from the right renal cortices (Online Figure II). In kidney sections from these mice, the intensity and extension of YFP signals at the JG area were lower in the right and higher in the left kidneys (Figure 2A). These results suggest that with high perfusion pressure, expression of the YFP gene decreased to undetectable levels in some renin cells, whereas with low perfusion pressure, new cells turned on the expression of YFP and renin. Furthermore, the data indicate the 4.9 kb of DNA sequence at the 5' *Ren1* regulatory region is sufficient to confer responses to changes in perfusion pressure.

We performed RNA-seq using YFP-positive renin cells from kidneys of 3 mice with AoCo and 2 mice with sham surgeries. Differential gene expression profiling using the paired analysis to compare gene expression between the right and left kidneys from the AoCo group revealed 202 differentially expressed genes (DEGs) (Figure 2B, Online Figure III-A, Online Table I, II). We confirmed the difference in expression of those newly-detected genes in renin cells subjected to AoCo using ISH (Online Figure IV). Of those DEGs, 94 were expressed higher in the right, and 108 were higher in the left kidneys (Figure 2B). In agreement with the above qRT-PCR and ISH data, *Ren1* expression in renin cells was higher in the left than in the right kidney. This shows that in addition to an increase in the number of renin-expressing cells (more YFP-positive cells), there was also an increase in *Ren1* expression by these cells in response to low perfusion pressure. Because YFP-positive cells

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are all renin-expressing cells, as expected, *Ren1* was the most highly expressed in both high and low perfusion pressure conditions (Online Figure III-A). *Kiss1*, which is located close to *Ren1* on chromosome 1, showed higher expression in renin cells from kidneys with low perfusion pressure and had the smallest *P*-value among DEGs (Figure 2B, Online Figure IV-C), although the absolute value of the expression was much lower than *Ren1* (Figure 2C). This is consistent with previous reports that *KISS1* and *REN* in humans exhibit similar expression patterns[6]. *Akr1b7*, a previously established renin cell marker gene[7], was also highly expressed in renin cells, and its expression levels were significantly different between cells from the right and left kidneys (Figure 2C), further confirmed by ISH (Online Figure V). We also detected 87 and 122 DEGs from high vs. normal perfusion pressure and normal vs. low perfusion pressure, respectively (Online Figure VI).

The identified DEGs by paired analysis from the AoCo group clearly separated the samples from the right and left kidneys (Figure 2D). Furthermore, when we included the renin cells from mice with sham surgeries, the DEGs also showed a clear separation of the samples according to the perfusion pressure with the sham group placed in between the ones from the right and left kidneys from the AoCo group (Online Figure III-B). Principal component analysis (PCA) also clearly segregated the samples according to changes in perfusion pressure (Figure 2E). The top principal component (PC1) represented more than half of the variances. PC1 was strongly associated with the perfusion pressure, whereas PC2 showed the differences between each mouse. We identified genes contributing to PC1 that are particularly important for the regulation of renin cells in response to changes in perfusion pressure, including *Ren1* (Figure 2E). The gene with the smallest *P*-value with high perfusion pressure, *serine protease inhibitor Kazal-type 8 (Spink8)*, may inhibit yet undefined proteases that convert inactive prorenin to active renin[8]. Genes of vacuolar ATPase were significantly higher with high perfusion pressure (Online Figure IV-A, VII). The vacuolar ATPase is associated with (pro)renin receptor[9] and related to autophagy[10] that may play a role in removing excessive renin in renin cells exposed to high perfusion pressure. Of genes upregulated with low perfusion pressure, *stromal cell-derived factor 2-like 1* (*Sdf2l1*)[11] (Online Figure IV-E) and *mesencephalic astrocyte-derived neurotrophic factor* (*Manf*)[12] are related to endoplasmic reticulum stress, and *aldehyde dehydrogenase family 7, member A1* (*Aldh7a1*) (Online Figure IV-B) is related to osmotic stress[13]. These changes likely reflect the transformation of renin cells to an endocrine phenotype in response to intracellular stress[14]. *E2F transcription factor 3* (*E2f3*) (Online Figure IV-F), a member of the E2F family which was increased with low perfusion pressure, may have direct and/or indirect effects on renin expression[15].

Gene Ontology (GO) enrichment analysis of biological processes identified several biological pathways related to changes in perfusion pressure (Figure 2F). As expected, pathways associated with BP regulation by the renin-angiotensin system were among the top biological pathways encountered. In addition, pathways for the development and differentiation of muscle cells were detected, which might reflect the phenotypical switching between renin cells and smooth muscle cells of the renin lineage. Consistent with this, smooth muscle cell-related genes, such as *vitronectin* (*Vtn*)[16] (Online Figure IV-D) and *creatine kinase, mitochondrial* 1 (*Ckmt1*)[17] were higher in renin cells with high perfusion pressure. Vitronectin is an adhesive glycoprotein of the extracellular matrix (ECM) that

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promotes cell adhesion, migration, and proliferation through its binding to integrins[16]. Similarly, *talin2* (*Tln2*), which activates integrins[18], and *HEPACAM family member 2* (*Hepacam2*), which is involved in cell-ECM interactions with F-actin[19], were increased with high perfusion pressure. Further, the expression of cytoskeletal components was increased in renin cells under high perfusion pressure, raising the possibility that cytoskeletal remodeling[20] is an adaptation to changes in perfusion pressure.

The cAMP pathway is crucial for the control and maintenance of renin-expressing cells[4]. *Neuromedin B* (*Nmb*)[21] and *Vip* (vasoactive intestinal polypeptide)[22], which increase intracellular cAMP, were upregulated with low perfusion pressure. A gap junction protein, *Gja5* encoding connexin 40, which is associated with *Ren1* expression[23], was significantly higher with low perfusion pressure (Online Table II). These changes support a phenotype switch from contractile to endocrine in response to decreased perfusion pressure.

Overall, we identified novel genes in renin cells that are regulated by changes in perfusion pressure. Renin cells are able to sense mechanical signals and respond transcriptionally by inhibiting or promoting the endocrine phenotype. It would be interesting to examine whether these phenotypic changes are manifested earlier in response to either activation or suppression of the baroreceptor mechanism. It is likely that chromatin events occur rapidly after changes in perfusion pressure and get firmly established with time.

### Mechanical stimuli affect Ren1 expression in vitro.

To test the direct effects of changes in pressure or mechanical stimulation on renin cells, we performed novel *in vitro* experiments using pneumatic chambers that can change the pressure applied to renin cells in culture (Figure 3A). We used our renal afferent arteriolar smooth muscle cell line (CFP/YFP cells) derived from mice where cyan fluorescent protein (CFP) is a renin lineage reporter[4] and YFP marks cells that actively express renin[24]. These cells express *Ren1* mRNA when stimulated by cAMP + IBMX, although its expression without stimulation is very low. We continuously applied pneumatic pressure of 80 mmHg and 250 mmHg to the cells and harvested the cells 72 hours after the treatment. By applying the pneumatic pressure to the renin cells, the expression of *Ren1* induced by cAMP + IBMX was significantly inhibited in a pressure-dependent manner (Figure 3B). Renin protein secretion also decreased by the application of pressure (Figure 3C). Staining for F-actin and α-smooth muscle actin confirmed a significant increase in the intensity of cytoskeletal actin filament in the cells exposed to pneumatic pressure (Figure 3D). This result suggests that as the cells decrease renin expression, they become more contractile and shift towards a myocrine phenotype in response to mechanical stimuli.

We also tested the influence of a different type of mechanical stimulus on renin cells using a magnetic force (Figure 3E). Fibronectin is the major ligand for integrin β1 which is highly expressed in renin cells[25]. Taking advantage of this ability of fibronectin to bind integrins, we applied fibronectin-coated magnetic beads to the CFP/YFP cells, which resulted in decreased *Ren1* mRNA expression. The expression of *Ren1* was further inhibited when we applied tensional force to the cells by placing a permanent magnet above the cells (Figure 3F). Overall, these results indicate that renin cells respond directly to mechanical stimuli with changes in the expression of the renin gene and concomitant changes in renin release.

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## Operation of the renal baroreceptor is mediated by integrin β1 transduction in response to changes in perfusion pressure into renin cells.

We then investigated the core components of the mechanotransduction mechanism in renin cells which regulate the changes in gene expression by perfusion pressure. The results of RNA-seq and *in vitro* magnetic force experiments suggested the involvement of integrins. Integrins are a large family of heterodimeric transmembrane glycoproteins that function in both cell-ECM and cell-cell adhesion and are involved in the bi-directional flow of signals from the extracellular environment to the cell interior[26]. Integrin dimers are composed of distinct α and β subunits. Our RNA-seq data showed that the integrin β1 gene (*Itgb1*) was the most highly expressed β integrin in renin cells during high, normal, and low perfusion pressure conditions (Online Figure VIII-A). And the integrin αV gene was the highest in the α subfamily (Online Figure VIII-B). By immunoprecipitation with an integrin β1 antibody following Western blot, we found integrin αV and α5 were present in the renin cells, forming heterodimers with integrin β1 (Online Figure VIII-C).

To test whether integrin β1 plays a role in renin cells in response to changes in perfusion pressure, we used a conditional knockout mouse model of integrin β1 in cells of the renin lineage (*Itgb1cKO*), which we recently generated[25] (Figure 4A). We performed AoCo surgeries on *Itgb1cKO* mice at 4 weeks of age. BP after three days of AoCo in *Itgb1cKO* mice was significantly lower than in control mice (Online Figure IX). When subjected to AoCo, the decrease in *Ren1* mRNA expression (by qRT-PCR) in the right kidneys and the increase in *Ren1* mRNA in the left kidneys were significantly blunted in *Itgb1cKO* mice when compared to controls (Figure 4B). ISH for *Ren1* mRNA showed that *Ren1* expression did not change in response to the AoCo in the *Itgb1cKO* mice when compared to control or sham groups (Figure 4C). These data suggest that integrin β1 in renin cells may represent one of the first steps in a mechanotransduction mechanism in response to changes in perfusion pressure, and without β1 integrin, the renin cells cannot respond appropriately to changes in perfusion pressure.

## Lamin A/C conveys mechanical stimuli to chromatin via a nuclear mechanotransduction mechanism.

The nuclear lamina mediates the transmission of mechanical forces from the cytoskeleton to the nucleus[27]. Lamins are the major architectural proteins of the nucleus and compose a filamentous protein network underneath the inner membrane that connects to the chromatin[28,29]. Nuclear lamins are divided into A and B types on the basis of structural and protein features and expression patterns[30]. The *Lmna* gene, which encodes lamin A and its splice variant lamin C, is expressed at high levels in renin cells regardless of changes in perfusion pressure, whereas the expression of *Lmnb1* and *Lmnb2* that encode lamin B is very low (Online Figure X-A). Analyzing the data of our chromatin immunoprecipitation sequencing[24], we found that the *Lmna* gene locus is associated with one of the 91 super-enhancers specific for renin cells, and it ranked in the fifteenth position for H3K27ac enrichment in recruited native renin cells (Online Figure X-B). With ATAC-seq, we found that the *Lmna* gene locus is epigenetically active, shown by its open chromatin signals in the several physiological stages of the native renin cells (Online Figure X-C). Hence, lamin A/C is prominent in renin cells and may be one of the crucial components of the nuclear

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mechanotransducer in controlling the expression of the renin gene and the identity and renin cell fate.

To investigate the role of lamin A/C in renin cells, we generated mice with conditional deletion of the *Lmna* gene in cells of the renin lineage (*LmnacKO*) by crossing *Ren1*$^{dCre}$ mice[31] and *Lmna* floxed mice[32] (Figure 5A). We confirmed the deletion of the *Lmna* gene using fluorescence ISH for *Lmna* mRNA and *Ren1* mRNA (Figure 5B) and immunohistochemistry (Online Figure XI-A) and immunofluorescence staining (Figure 5C) for lamin A/C and renin. *Lmna* mRNA and lamin A/C were clearly observed in renin expressing cells at JG areas in the control mice, whereas *LmnacKO* mice did not show signals in renin expressing cells (Figure 5B, C, Online Figure XI-A). *LmnacKO* mice were born at the expected mendelian ratio, developed normally, and showed no alterations in renal function, renin expression, or kidney morphology under basal conditions (Online Figure XI-B-F). To test how renin cells lacking lamin A/C react to changes in perfusion pressure, we performed AoCo on *LmnacKO* mice (Figure 5A). AoCo induced significant differences in *Ren1* mRNA between the right and left renal cortices in both control and *LmnacKO* mice. Simultaneous BP measurements from the carotid and femoral arteries in these mice showed significant differences between BPs above and below the coarctation in both the control and *LmnacKO* mice. However, the pressures were not different between the control and *LmnacKO* mice (Online Figure XII), suggesting that the kidneys in the *LmnacKO* mice were exposed to the same BP changes as the control mice. However, the expression changes shown by the left/right ratio of *Ren1* mRNA were significantly lower in *LmnacKO* mice than those of control mice (in both female and male mice) (Figure 5D, E). ISH showed that the decrease in *Ren1* mRNA at JG areas in the right kidneys and the increase in the left kidneys were milder in the *LmnacKO* mice than those of the control mice, respectively (Figure 5F). Taken together, *LmnacKO* mice showed a diminished ability to respond to changes in perfusion pressure resulting from AoCo, underscoring the role of lamin A/C in transcriptional regulation of the renin gene in response to changes in perfusion pressure.

### Lamin A/C regulates Ren1 expression in response to mechanical stimuli in vitro.

To further investigate the role of lamin A/C in the regulation of *Ren1* expression, we knocked out the *Lmna* gene in the CFP/YFP cells using CRISPR-Cas9 (Figure 6A). We targeted the exon 2 of *Lmna* to knockout all the transcript variants of the gene (Online Figure XII-A). Following CRISPR-Cas9 ribonucleoproteins transfection, we obtained an efficient knockout of *Lmna* (*LmnaKO*), confirmed by PCR and Sanger sequencing of genomic DNA from the cells and qRT-PCR for *Lmna* mRNA (Online Figure XIII-B-D). By immunocytochemistry, only 4.92 ± 2.20% of the renin cells showed the signals of lamin A/C in the *LmnaKO* cell pools (Figure 6B), indicating sufficient efficiency of the knockout (~95%) for further analysis.

*LmnaKO* renin cells showed lower expression of *Ren1* when compared to the control renin cells upon stimulation with cAMP + IBMX (Figure 6C). The application of pneumatic pressure showed significant reductions of *Ren1* mRNA (by qRT-PCR) in control renin cells. In contrast, the *LmnaKO* renin cells did not show changes in *Ren1* expression (Figure 6D). Next, by fluorescence ISH, *Ren1* expression was also lower in *LmnaKO* renin cells

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compared to controls (Figure 6E). Whereas control renin cells showed a marked decrease in the *Ren1* expression in response to pneumatic pressure, in *LmnaKO* renin cells, the reduction was less pronounced (Figure 6E, Online Figure XIV). These results demonstrate that *in vivo* and *in vitro*, lamin A/C plays an essential role in renin cells in the control of *Ren1* expression in response to external mechanical stimuli.

### Lamin A/C regulates chromatin organization in renin cells.

To test the effect of *Lmna* knockout on chromatin structure, we performed ATAC-seq in the CFP/YFP cells 7 days after the CRISPR-Cas9 reaction. Using 2 replicates of control cells and 2 replicates of *LmnaKO* cells, we identified 81,394 and 78,833 accessible chromatin regions in control cells and *LmnaKO* cells, respectively. Control and *LmnaKO* cells shared a similar overall distribution of accessible chromatin (Figure 7A). However, the PCA of the accessible chromatin loci showed clear separation of control and *LmnaKO* cells, which was supported by the Pearson correlation coefficient (Figure 7B). Although the cells tested were not stimulated by cAMP +IBMX, the region upstream of the *Ren1* gene was open in both cases (Figure 7C). The position of the peaks was the same as previously shown[24], and there was no difference between the control and *LmnaKO* cells at this locus. These results suggest that the *Ren1* gene locus in these cells is poised without stimulation and is activated by cAMP with the chromatin open. With differential accessible analysis, we identified 62 and 68 peaks that are significantly different between the control and *LmnaKO* cells (Online Table V). GO enrichment analysis of biological processes identified that the peaks that were changed by *LmnaKO* were related to cellular development, including kidney and nephron (Figure 7D). These data indicate that lamin A/C in renin cells interact with chromatin to regulate its organization, and loss of lamin A/C leads to changes in chromatin accessibility with unanticipated changes in developmental fates.

The ATAC-seq result suggests that loss of *Lmna* in renin cells induces partial chromatin structural changes under basal conditions. However, with the current technology, it is not possible to perform ATAC-seq with cells receiving mechanical stimuli, as cells are released from stimulation during the procedure. Therefore, to investigate the role of lamin A/C in chromatin organization in renin cells that are receiving mechanical stimuli, we performed immunocytochemistry for epigenetic marks involved in chromatin configuration on cells immediately fixed after the pneumatic pressure treatment. The *LmnaKO* renin cells showed dysmorphic nuclei, with unsmooth, irregular nuclear peripheries, compared to the control cells even without pneumatic pressure (Figure 7E). Staining for several histone modification marks showed heterogeneous distributions of the histone marks and heterochromatin. Particularly, histone H3 lysine 9 dimethylated (H3K9me2) chromatin at the nuclear lamina was not distributed equally in the *LmnaKO* renin cells, consistent with previous reports of human cells with *LMNA* mutation[33]. These nuclear abnormalities were enhanced by applying pneumatic pressure (Figure 7E, Online Figure XV). These data indicate that lamina-chromatin interactions are disrupted by *LmnaKO*, which induces failure to adjust transcription of the genes, including *Ren1*, appropriately by changes in perfusion pressure.

Taken together, we identified that lamin A/C is essential to maintain the chromatin structure in renin cells, which is essential for nuclear mechanotransduction. Loss of lamin A/C in

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renin cells prevents their ability to respond appropriately to mechanical stimuli by affecting gene transcription. It is likely that loss of *Lmna* prevents renin cells from modifying the chromatin in response to stimuli and thus blocks upregulation of renin gene expression.

## DISCUSSION

In 1957, Tobian and coworkers postulated the existence of a pressure sensing mechanism that controlled renin release and thus BP homeostasis[3]. In this tightly regulated mechanism, high perfusion pressure suppresses, whereas low perfusion pressure stimulates renin release from renin cells[34-36]. The sensing mechanism was denominated the renal "baroreceptor," and it is believed to regulate both renin secretion and transcription of the renin gene. Both effects, release and transcription are likely to operate in concert, although this needs to be studied in detail. The structure and location of the baroreceptor have remained an enigma for over sixty years[37]. In this study, we focus our efforts on investigating the effects of mechanical/pressure changes on renin gene expression. We show here that the pressure sensing mechanism resides within the renin cells, and it is a complex mechanotransducer: changes in pressure are sensed by integrin β1, likely dimerizing with αV or α5, and transmitted to the cell's nucleus via lamin A/C which induces chromatin architectural changes that ultimately control *Ren1* expression and the identity of the renin cells (Figure 8).

The ability of a cell to respond to mechanical forces is crucial for numerous biological processes. It has become evident in recent years that external mechanical forces induce changes in nuclear structure and composition, chromatin organization, and gene expression[28]. Although it is difficult to completely recreate the physical conditions of the cells in the afferent arterioles, to study mechanotransduction in renin cells, we used novel *in vivo* and *in vitro* approaches. The *in vitro* experiments we performed have not previously been attempted and represent a first step in understanding how physical stimuli modify the expression of the renin gene and the overall phenotype of the cells. With these *in vitro* experiments, application of pneumatic pressure and magnetic forces -via fibronectin-coated magnetic beads, we found that expression of the renin gene is controlled by a direct mechanical stimulus. To extend these findings *in vivo*, we established a surgical model of AoCo between the roots of both right and left renal arteries, which allowed the comparison between cells exposed to high and low perfusion pressure in the same animal, thus eliminating the possibility of spurious changes due to inter-animal variability or genetic background, a clear advantage when compared to traditional rat surgery models[38,39].

Several cell surface protein assemblies have been characterized that can sense physical and chemical signals[20]. In this study, we identified integrin β1 as one of the critical components of sensing and transduction of extracellular mechanical signals into renin cells (Figure 8). Integrins are heterodimeric cell surface receptors ensuring the mechanical connection between cells and the ECM, conveying externally applied forces to the intracellular complex[40]. It has been shown that forces are transmitted from the cell surface to the nucleus through integrins, the actin cytoskeleton, the LINC complex, and then lamin–chromatin interactions, which in turn regulate chromatin architecture and gene transcription[41]. Thus, extracellular forces exerted on the nucleus by the cytoskeleton may influence nuclear morphology, chromatin organization, and gene transcription[42]. Low responsiveness of the

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renin gene to AoCo in *Itgb1cKO* mice suggests that an integrin with a β1 subunit is one of the critical sensors in renin cells that perceive changes in perfusion pressure and regulate *Ren1* expression.

Mechanotransduction transmits mechanical signals to the nucleus to regulate gene expression[43]. We found that lamin A/C inside the nuclei is essential to regulate *Ren1* expression in renin cells in response to changes in perfusion pressure (Figure 8). Whereas in animals without AoCo, *Ren1* expression in *LmnacKO* mice was not different from controls, the reactivity to AoCo for *Ren1* expression was significantly impaired in the *LmnacKO* mice. Further, *in vitro experiments* showed a similar effect of lamin A/C on cells exposed to a mechanical stimulus. Together, these results demonstrate the novel finding that lamin A/C in renin cells directly controls *Ren1* expression. The nuclear lamina anchors to the inner nuclear membrane, DNA, and chromatin[44]. Lamins B1 and B2 are usually localized at the peripheral nuclear lamina, where they interact with extended chromatin domains called lamina-associated domains (LADs)[20,27]. However, LADs are not static, and unlike lamin B which is typically associated with heterochromatin, lamin A/C is associated with gene-rich and transcriptionally active euchromatic regions[44,45]. Lamin A/C interacts with chromatin through LADs and influences gene expression in a cell type-specific manner[46]. Hence, it is possible that lamin A/C attaches to specific genomic loci in renin cells and mechanically changes the three-dimensional structure of the chromatin related to *Ren1* expression. Our ATAC-seq and immunocytochemistry for several histone marks on *LmnaKO* cells indicated that the chromatin structure in renin cells is affected by deletion of lamin A/C. Recently, we found that renin cells possess unique chromatin states characterized by open chromatin and the presence of super-enhancers that convey the physiologic status of the organism and are responsible for the transformation of renin cell descendants to the renin phenotype[24]. These findings suggest that the nuclear mechanotransduction in renin cells induces chromatin architectural changes and formation of the super-enhancers at the renin gene. Further studies will be required to explain how lamin A/C regulates *Ren1* expression in renin cells through chromatin modification initiated by changes in perfusion pressure.

In addition to changes in the expression of *Ren1*, our RNA-seq data from sorted renin cells showed clear differences in the repertoire of genes expressed by renin cells obtained from the right and left kidneys exposed to high or low perfusion pressure, respectively. Interestingly, cells exposed to low perfusion pressure expressed a variety of genes directly related to the endocrine phenotype (*Ren1, Akr1b7, Sdf2l1*), whereas cells exposed to high perfusion pressure maintained a contractile phenotype (*Vtn, Ckmt1*) while diminishing their endocrine characteristics (*Spink8, Atp6v1g3*). We have previously shown that in response to physiological challenges, renin cells possess the innate ability or plasticity to switch phenotypes from a smooth muscle-contractile phenotype to an epitheloid-granular phenotype[1]. We show here that the memory of the renin phenotype is controlled *in vivo* and *in vitro* by a nuclear mechanotransducing mechanism that controls the identity and plasticity of renin cells.

In summary, we elucidated the structure and location of the renal baroreceptor, a pressure sensing and mechanotransducing mechanism in renin cells that conveys mechanical signals

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directly to the chromatin to control renin expression and renin cell identity to maintain homeostasis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Nonstandard Abbreviations and Acronyms:

| | |
|---|---|
| **AoCo** | Aortic coarctation |
| **ATAC-seq** | Assay for Transposase-Accessible Chromatin using sequencing |
| **BP** | Blood pressure |
| **CFP** | Cyan fluorescent protein |
| **DEGs** | Differentially expressed genes |
| **ECM** | Extracellular matrix |
| **GO** | Gene Ontology |
| **ISH** | *in situ* hybridization |
| **JG** | Juxtaglomerular |
| **LADs** | Lamina-associated domains |
| **PC** | Principal component |
| **PCA** | Principal component analysis (PCA) |
| **qRT-PCR** | Quantitative reverse transcription PCR |
| **YFP** | Yellow fluorescent protein |

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## NOVELTY AND SIGNIFICANCE

**What Is Known?**

- The existence of a pressure sensing-mechanism (the baroreceptor) that controls renin release has been postulated for a long time. However, the nature and location of this baroreceptor and how it controls renin expression are unclear.
- Renin cells synthesize and release renin to the circulation in response to a decrease in perfusion pressure.
- By increased perfusion pressure, renin cells decrease renin release preventing the development of hypertension.

**What New Information Does This Article Contribute?**

- The baroreceptor is located in the renin cell and consists of a nuclear mechanotransducer that controls renin expression.
- Changes in perfusion pressure are sensed by integrin β1 and transmitted to the renin cell's nucleus via lamin A/C, which induces chromatin architectural changes that control the expression of the renin gene.

Renin cells are crucial for survival. They are sensors with the exquisite ability to detect changes in arterial blood pressure and respond to those changes with exact amounts of renin released to the circulation to maintain blood pressure and fluid-electrolyte homeostasis. The location and nature of this unique pressure-sensing structure have remained unknown since it was originally suggested as a "baroreceptor" over sixty years ago. Our studies show that the enigmatic baroreceptor is a novel nuclear mechanotransducer that resides in the renin cells per se and is responsible for the sensing and transmission of extracellular physical forces directly to the chromatin to regulate renin gene expression, circulating renin, and homeostasis.

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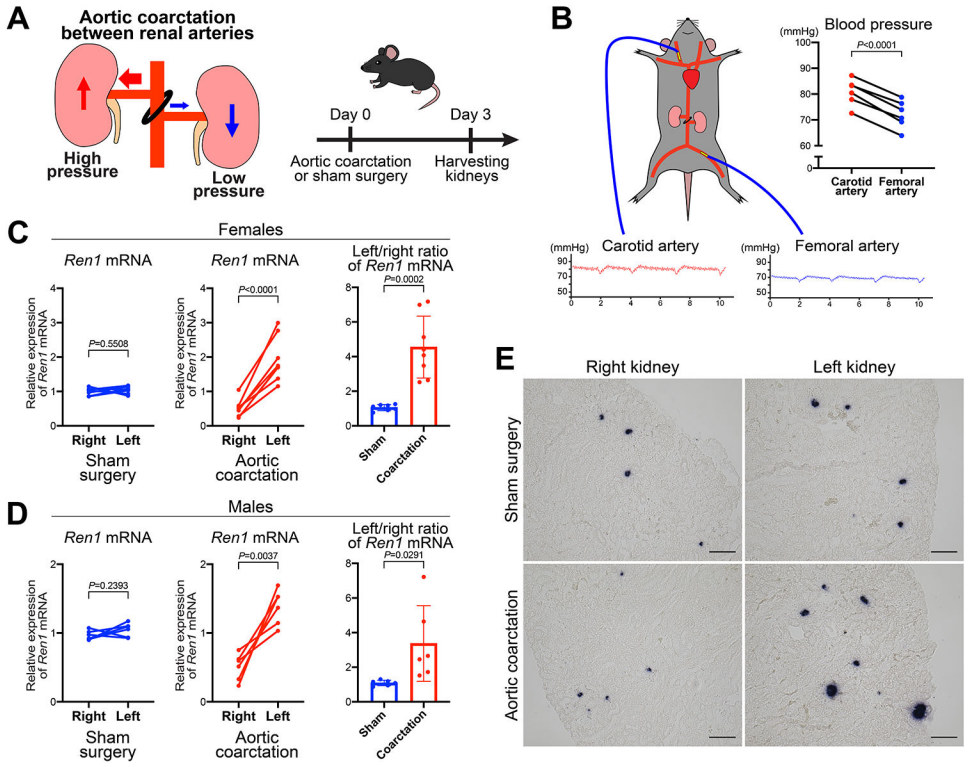


**Figure 1. Changes in perfusion pressure induce modifications in *Ren1* expression.**

**A,** Schematic of the aortic coarctation (AoCo) surgical model. The ligation between the roots of the right and left renal arteries results in an increase in perfusion pressure in the right kidney and a decrease in the left kidney. Kidneys were harvested 72 hours after the AoCo or sham surgeries. **B,** Simultaneous blood pressure (BP) measurement under anesthesia from the carotid and femoral arteries of mice with AoCo. The BP was significantly different between upper arteries (carotid arteries) and lower arteries (femoral arteries) of AoCo ($n$=6, paired t-test). **C,** Changes in *Ren1* expression in kidneys subjected to AoCo in female mice by quantitative reverse transcription PCR (qRT-PCR) showed a significant difference in *Ren1* mRNA between the right and left renal cortices from mice with AoCo ($n$=8, paired t-test), whereas *Ren1* mRNA was not different between right and left renal cortices from mice with sham surgeries ($n$=7, paired t-test). With AoCo, the left/right ratio of *Ren1* mRNA was significantly higher than the one of sham surgeries (Student's t-test). **D,** Changes in *Ren1* expression in kidneys subjected to AoCo in male mice by qRT-PCR showed a significant difference in *Ren1* mRNA between the right and left renal cortices from mice with AoCo ($n$=6, paired t-test), whereas *Ren1* mRNA was not different between right and left renal cortices from mice with sham surgeries ($n$=6, paired t-test). With AoCo, the left/right ratio of *Ren1* mRNA was significantly higher than the one of sham surgeries (Student's t-test). **E,** *In situ* hybridization for *Ren1* mRNA in the kidneys. The intensity and extension of the *Ren1* mRNA signals at the juxtaglomerular areas were not different between the right and left kidneys from mice with sham surgery. With AoCo, signals in right kidneys were decreased, and signals in left kidneys were significantly increased, compared to sham surgeries. Scale bar, 100 μm. All data are reported as means ± standard deviation. *$P$<0.05, **$P$<0.01, ***$P$<0.001, ****$P$<0.0001.

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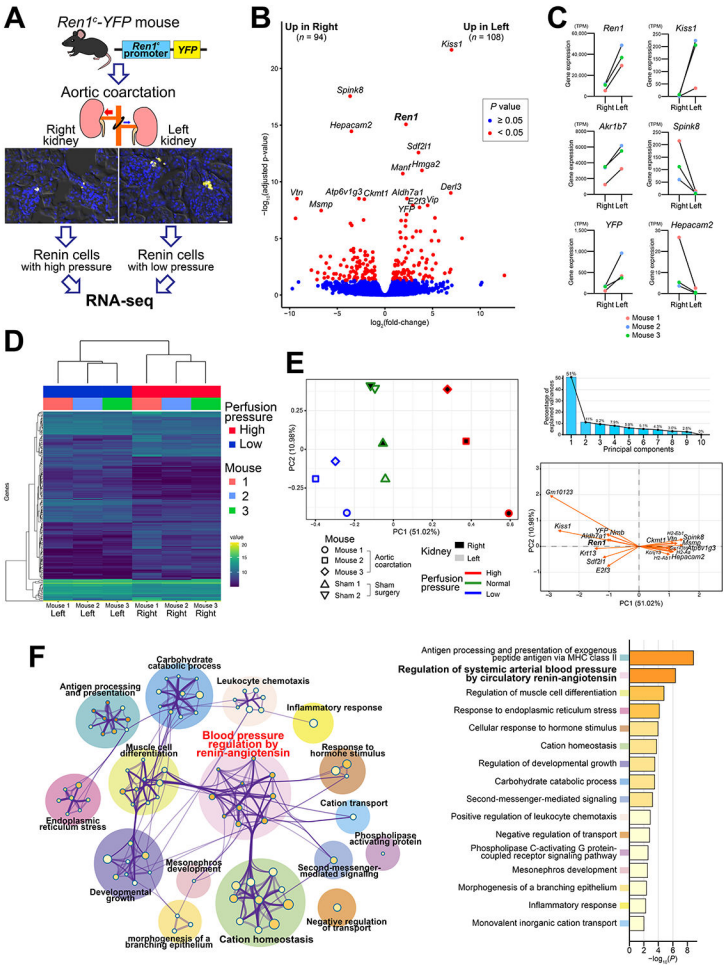


**Figure 2. Renin cells change their transcription programs in response to changes in perfusion pressure.**

**A,** RNA-seq of renin cells from the kidneys subjected to aortic coarctation (AoCo) surgery. YFP-positive cells from *Ren1*$^{c}$*-YFP* mice were isolated using fluorescence-activated cell sorting. Each kidney cortex was processed separately. Scale bar, 20 μm. **B,** Volcano plot of RNA-seq analysis. The 202 differentially expressed genes (DEGs) between renin cells in the right kidneys and left kidneys were shown in red. **C,** Representative genes that showed differential expression between renin cells in the right and left kidneys. Data are shown in transcripts per million (TPM). **D,** Heatmap using the expression levels of DEGs showed clear segregation of renin cells in the right and left kidneys, indicating marked differences in gene expression in response to changes in perfusion pressure. **E,** Principal component analysis (PCA) by the DEGs of renin cells from kidneys subjected to AoCo and sham surgeries. PC1 clearly segregated renin cells with low, normal, and high perfusion pressure. PC1 represented 51.02 % of the variances. The top 20 genes that contributed to PC1 were shown in the variable correlation plot. **F,** Enrichment analysis using Gene Ontology with the DEGs between renin cells in the right and left kidneys. The enriched categories were shown with the network analysis.

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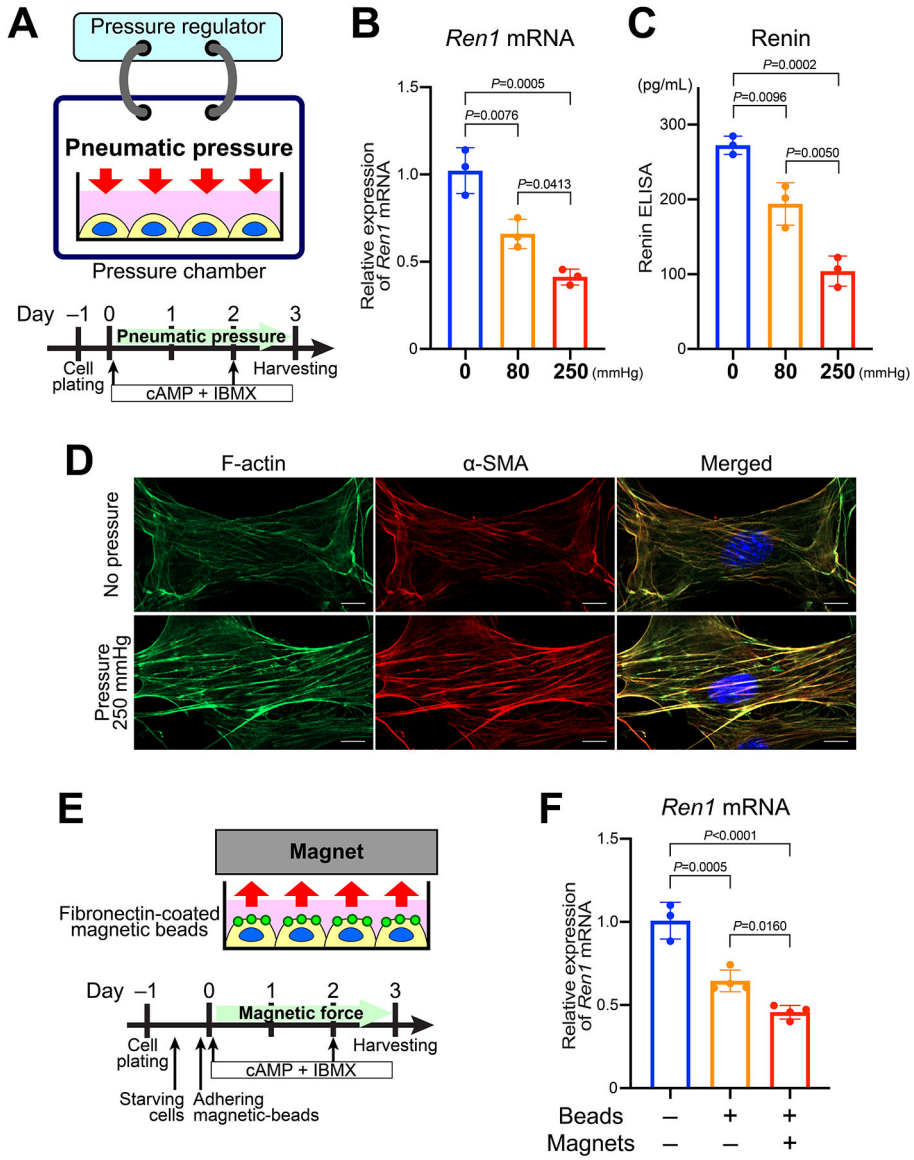


**Figure 3. Mechanical stimuli to renin cells *in vitro* inhibit *Ren1* expression.**

**A,** Schematic of the pneumatic pressure model. We used renin cells stimulated by cAMP + IBMX. Cells were harvested after 3 days of stimulation and pressure application. **B,** Quantitative reverse transcription PCR (qRT-PCR) showed a decrease in *Ren1* mRNA expression in a pressure-dependent manner ($n$=3, one-way ANOVA followed by Tukey's multiple comparison test). The data are representative of three independent experiments. **C,** ELISA for renin in the culture medium showed that secretion of renin protein from renin cells also decreased in response to the pneumatic pressure ($n$=3, one-way ANOVA followed by Tukey's multiple comparison test). **D,** F-actin staining by phalloidin and immunocytochemistry for α-smooth muscle actin (α-SMA) showed the increased intensity of actin filaments in cultured renin cells subjected to pneumatic pressure. Scale bar, 10 μm. **E,** Schematic of the magnetic force model. Fibronectin-coated magnetic beads adhered to the cells of the renin lineage. The renin cell phenotype was induced by cAMP + IBMX, and

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tensile force was applied by placing permanent magnets above the cells. **F,** qRT-PCR showed a decrease in *Ren1* mRNA expression by applying fibronectin-coated magnetic beads. The *Ren1* mRNA expression decreased further when magnets were placed above the cells to apply the force ($n \geq 3$, one-way ANOVA followed by Tukey's multiple comparison test). The data are representative of three independent experiments. All data are reported as means ± standard deviation. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$.

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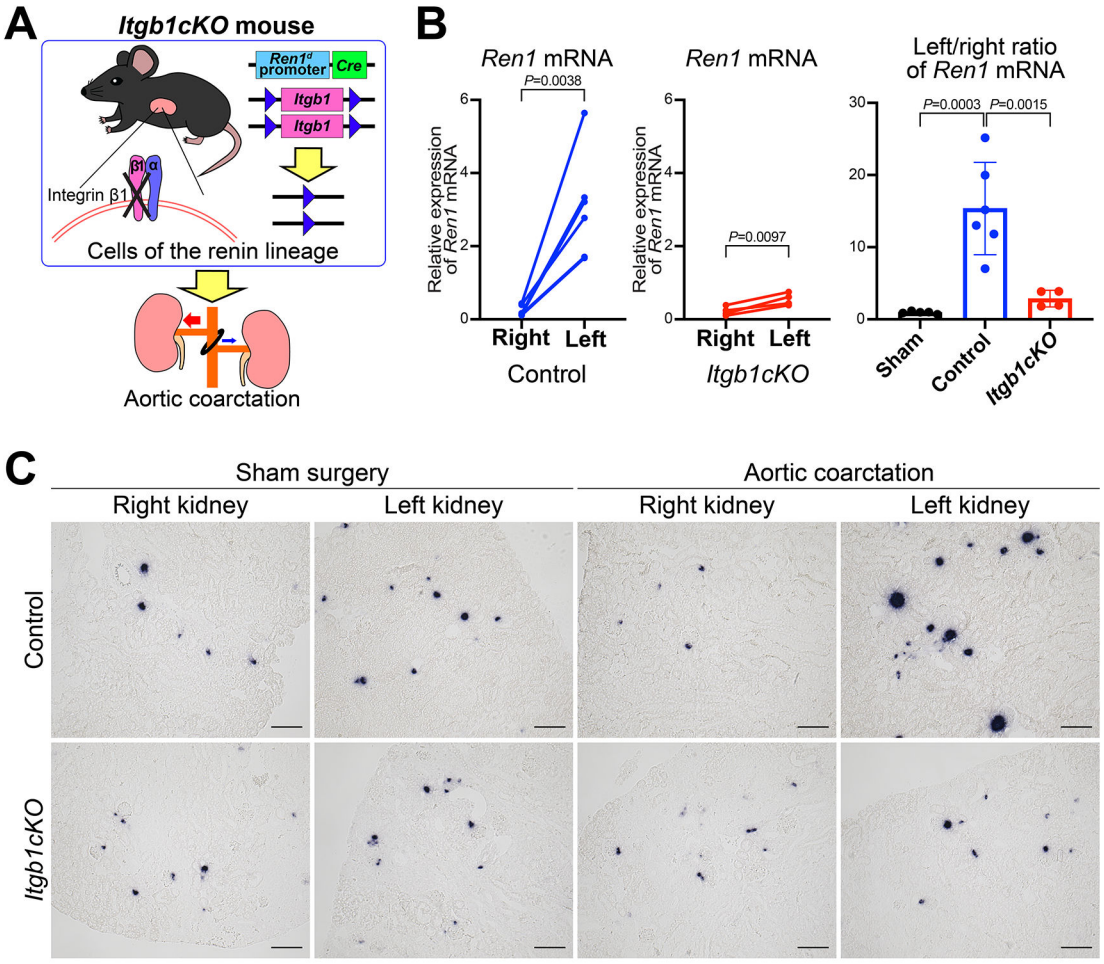


**Figure 4. *Itgb1* gene knockout in cells of the renin lineage inhibited the response to changes in perfusion pressure.**

**A,** Mice with conditional deletion of the *Itgb1* gene in cells of the renin lineage (*Itgb1cKO*) and control mice were subjected to aortic coarctation (AoCo). **B,** *Itgb1cKO* mice showed impaired responses in *Ren1* expression to changes in perfusion pressure. Quantitative reverse transcription PCR showed significant differences in *Ren1* mRNA between the right and left renal cortices from the control mice ($n$=6, paired t-test) and from the *Itgb1cKO* mice ($n$=4, paired t-test), respectively. The left/right ratio of *Ren1* mRNA of *Itgb1cKO* mice was significantly lower than the one of control animals (one-way ANOVA followed by Tukey's multiple comparison test). **C,** *in situ* hybridization for *Ren1* mRNA in the kidneys from *Itgb1cKO* mice subjected to the AoCo. For Itgb1cKO mice, the decrease in *Ren1* mRNA in the right kidneys and the increase in that in the left kidneys were milder than those in control mice, respectively. Scale bar, 100 μm. All data are reported as means ± standard deviation. **$P$<0.01, ***$P$<0.001.

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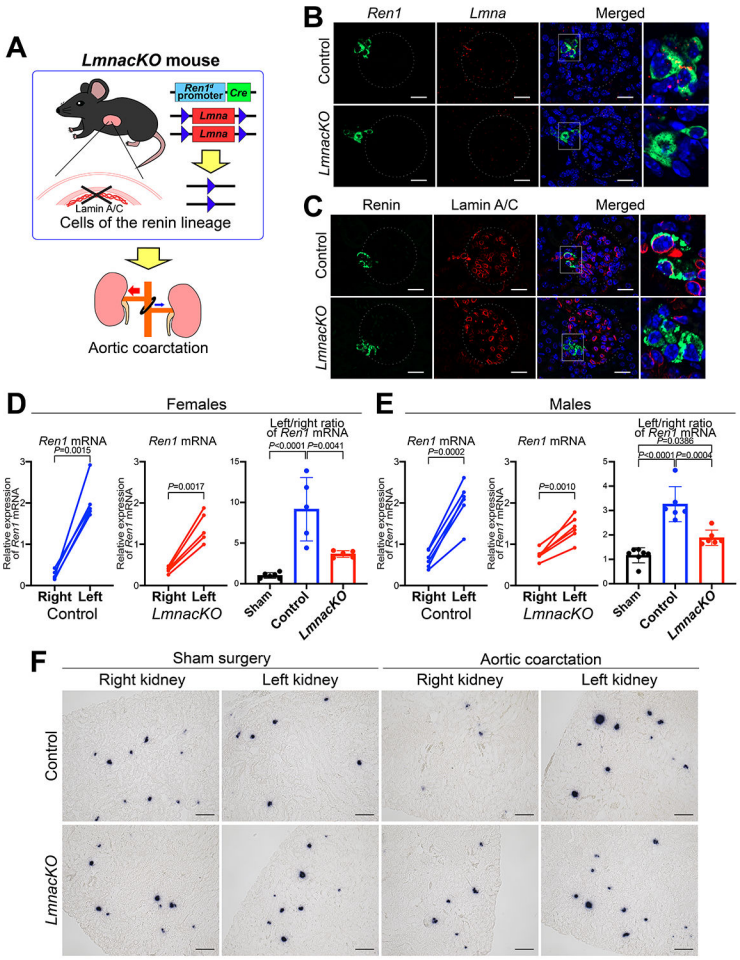


**Figure 5. *Lmna* gene knockout in cells of the renin lineage inhibited the response to changes in perfusion pressure.**

**A,** Mice with conditional deletion of the *Lmna* gene in cells of the renin lineage (*LmnacKO*) and their controls were subjected to aortic coarctation (AoCo). **B,** Fluorescence *in situ* hybridization (ISH) for *Ren1* mRNA (green), *Lmna* mRNA (red), and DAPI (blue). *Lmna* was highly expressed in the renin-expressing cells in the control mice. There was no *Lmna* expression in renin-expressing cells in *LmnacKO* mice. Dashed circles indicate glomeruli. Scale bar, 20 μm. **C,** Immunofluorescence staining for renin (green), lamin A/C (red), and Hoechst (blue). Lamin A/C was detected in the nuclei of renin cells in control mice and absent in the nuclei of renin cells in *LmnacKO* mice while preserved in non-renin cells. Dashed circles indicate glomeruli. Scale bar, 20 μm. *LmnacKO* showed impaired responses of *Ren1* expression to changes in perfusion pressure in both female (**D**) and male mice (**E**). Quantitative reverse transcription PCR showed significant differences in *Ren1* mRNA between the right and left renal cortices from control mice ($n$=5 for females and $n$=6 for males, paired t-test) and from *LmnacKO* mice ($n$=5 for females and $n$=6 for males, paired t-test). The left/right ratio of *Ren1* mRNA from *LmnacKO* mice was significantly lower than the one from control animals (one-way ANOVA followed by Tukey's multiple comparison test). **F,** ISH for *Ren1* mRNA in kidneys from *LmnacKO* mice subjected to the AoCo. In *LmnacKO* mice, the decrease in *Ren1* mRNA in the right kidneys and the increase in the left

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kidneys were milder than those from the control mice, respectively. Scale bar, 100 μm. All data are reported as means ± standard deviation. *$P$<0.05, **$P$<0.01, ***$P$<0.001, ****$P$<0.0001.

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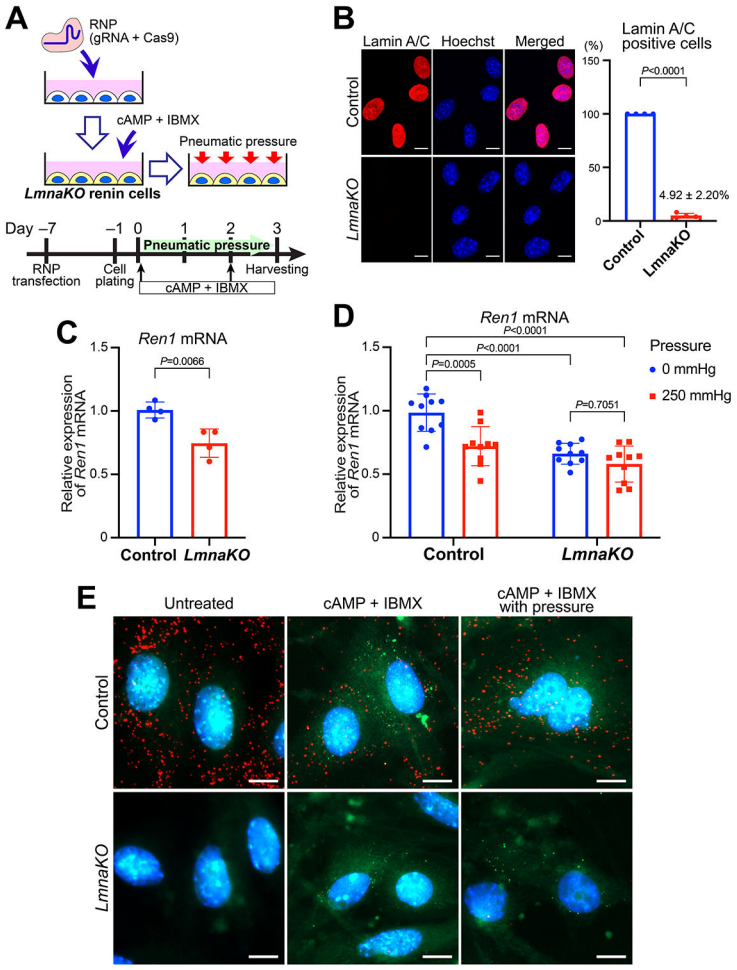


**Figure 6. Lamin A/C regulates the expression of *Ren1* in response to mechanical stimuli.**

**A,** Schematic of the knockout of the *Lmna* gene (*LmnaKO*) using CRISPR-Cas9. The ribonucleoproteins (RNPs), made with gRNAs for the exon 2 of *Lmna* and Cas9, were transfected to the renin lineage cells. Seven days after the transfection, cells were stimulated with cAMP + IBMX to regain the renin phenotype. Pneumatic pressure was applied as described above. **B,** Immunocytochemistry for Lamin A/C to measure the efficiency of the *Lmna* gene knockout. The percentage of the Lamin A/C positive cells was significantly lower in the *LmnaKO* renin cells ($n$=4, Student's t-test). Scale bar, 10 μm. Averages of three fields per experiment from four independent experiments were used. **C,** The *Ren1* mRNA expression in the *LmnaKO* renin cells induced by cAMP + IBMX was significantly lower than in control renin cells by quantitative reverse transcription PCR (qRT-PCR) ($n$=4, Student's t-test). The data are representative of three independent experiments. **D,** *LmnaKO* in cultured renin cells inhibited the decrease in *Ren1* expression by the pneumatic pressure. qRT-PCR showed significant reductions of *Ren1* mRNA in control renin cells subjected to pneumatic pressure, whereas there was no significant difference in *LmnaKO* renin cells ($n$=15, two-way ANOVA followed by Sidak's multiple comparison test). Data are from three independent experiments with 3–4 replicates per condition. **E,** Fluorescence *in situ* hybridization in cultured renin cells for *Lmna* mRNA (red), *Ren1* mRNA (green), and DAPI

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(blue). The *Ren1* expression (shown as green dots) was induced by cAMP + IBMX, and it was lower in *LmnaKO* renin cells compared to control. Control renin cells showed a decrease in the *Ren1* expression by the pneumatic pressure. *LmnacKO* renin cells did not affect *Ren1* mRNA expression in response to pneumatic pressure. Scale bar, 10 μm. All data are reported as means ± standard deviation. ***P*<0.01, ****P*<0.001, *****P*<0.0001.

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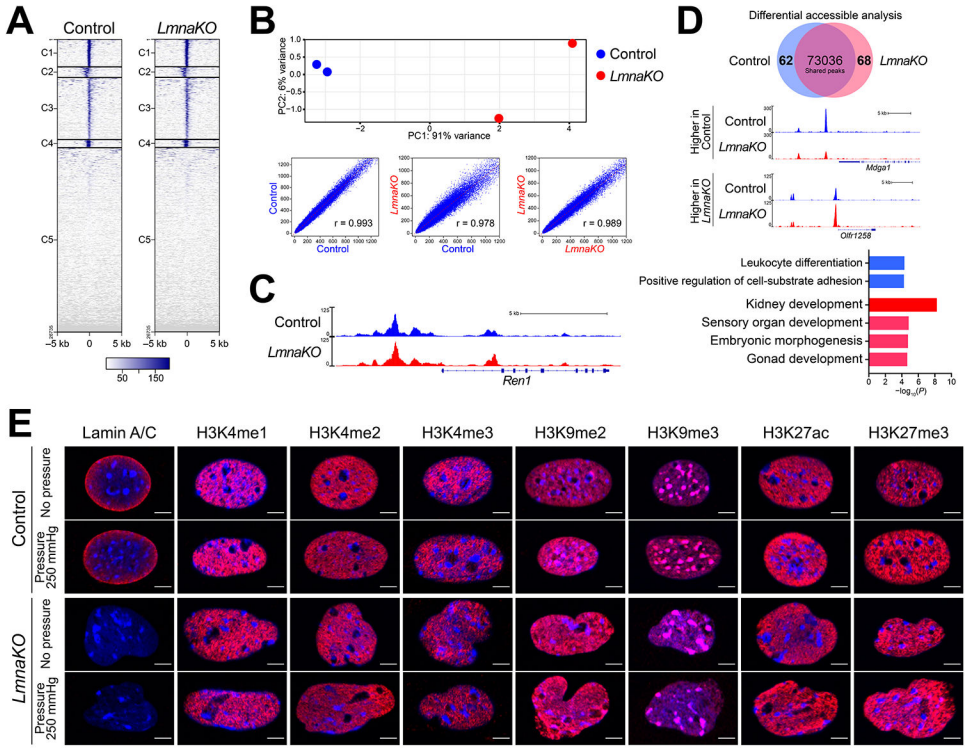


**Figure 7. Lamin A/C regulates chromatin organization in renin cells.**

**A,** Heatmaps showing normalized ATAC-seq signal intensity around the transcription start site (TSS) in control cells and cells with deletion of the *Lmna* gene (*LmnaKO*). **B,** *LmnaKO* cells showed a difference in accessible chromatin compared to controls. Principal component analysis (PCA) with identified ATAC-seq peaks in 2 replicates of each group and the Pearson correlation coefficient clearly separated samples of each group. **C,** Images of peaks of the accessible chromatin at the *Ren1* regulatory locus. **D,** The differential accessible analysis identified 62 and 68 peaks in the control and *LmnaKO* cells, respectively. The images show representative loci of the differential accessible regions. Gene Ontology (GO) analysis identified enriched categories on genes associated with peaks significantly higher in control (blue) and higher in *LmnaKO* (red). **E,** Immunocytochemistry for lamin A/C, H3K4me1, H3K4me2, H3K4me3, H3K9me2, H3K9me3, H3K27ac, and H3K27me3 shown in red and nuclear staining with Hoechst in blue in renin cells with or without *LmnaKO* subjected to pneumatic pressure. Scale bar, 5 μm.

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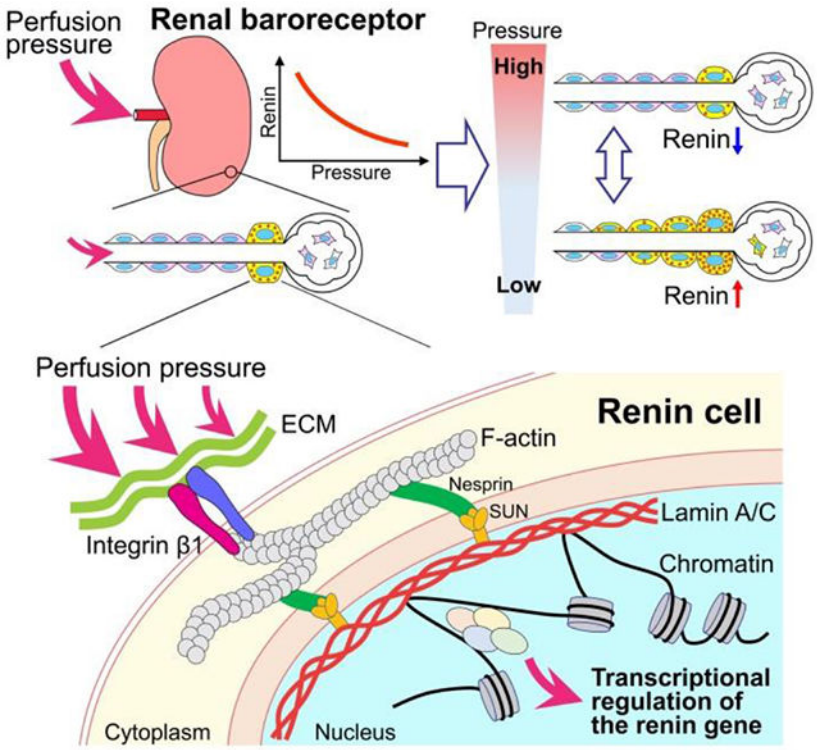


**Figure 8. The nuclear mechanotransduction mechanism in renin cells.**

DNA is connected to the extracellular environment through an array of proteins. The perfusion pressure change may be transmitted from the extracellular matrix (ECM) to the cytoskeletal elements by integrin and transduced to the lamin A/C at the inner nuclear membrane in renin cells, likely through F-actin, nesprin, and SUN domains. Lamin A/C may directly interact with chromatin and DNA and induce chromatin architectural changes to regulate the renin gene expression. Thus, changes in perfusion pressure transmit directly to the renin cells and the renin gene via a nuclear mechanotransduction mechanism. Intermediate molecules remain to be identified.