Hypertension

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Sex-Specific Exosome Cargo Reveals Potential New Mechanisms of Salt-Sensitive Hypertension

Vahideh Tarhriz,* Huijing Xia,* Ayushi Patel, Mortaza Eivazi, Amy Scarborough, Kevin Miao, Hasan Mir, Leila Abkhooie, Franck Mauvais-Jarvis, Eric Lazartigues

BACKGROUND: Hypertension is a multifactorial disease influenced by sex hormones, with notable sex-specific differences in its development and progression. Extracellular vesicles have emerged as important mediators in hypertension pathophysiology. This study aimed to investigate sex-specific extracellular vesicle–derived microRNA and protein profiles in deoxycorticosterone acetate-salt–induced hypertension.

METHODS: Male and female C57BL/6J mice underwent deoxycorticosterone acetate-salt treatment, with blood pressure monitored via telemetry and cardiac function assessed using echocardiography and invasive hemodynamics. Extracellular vesicles from plasma and cerebrospinal fluid were isolated and analyzed for microRNA (high-throughput RNA sequencing) and protein (liquid chromatography–mass spectrometry) content. To determine the contribution of sex hormones, gonadectomy was performed before deoxycorticosterone acetate-salt exposure. Hypothalamic and plasma samples were then used to validate key molecular findings.

RESULTS: Deoxycorticosterone acetate-salt treatment caused more severe hypertension, cardiac dysfunction, and mortality in males compared with females. Gonadectomy reduced hypertension and mortality in males but exacerbated them in females, confirming the protective effect of estrogens and the deleterious influence of androgens. Sex-specific extracellular vesicle–derived microRNA and protein expression profiles were identified, revealing 10 key regulatory microRNAs and highlighting potential regulatory axes such as miR (microRNA)-125b-5p/ACE (angiotensin-converting enzyme) 2, miR-1a-3p/G6PD (glucose-6-phosphate dehydrogenase), miR-410-3p/AT_1R (angiotensin II type 1 receptor), and miR-378a-5p/IRAP (insulin-regulated aminopeptidase). Gonadectomy altered expression patterns, supporting the hormone-dependent regulation of these microRNAs. Proteomic data showed renin-angiotensin system and diabetic cardiomyopathy pathway activation in hypertensive males. In silico and ex vivo analyses identified 25 microRNA-targeted genes, such as *G6pdx* and *IRAP*, reinforcing the role of sex hormone–sensitive microRNA-protein interactions.

CONCLUSIONS: This study highlights potential sex-specific microRNA networks in hypertension and proposes novel molecular targets for validation toward personalized, sex-tailored therapies. ***(Hypertension.* 2026;83:693–707. DOI: 10.1161/HYPERTENSIONAHA.125.25949.)** • **Supplement Material**.

Key Words: extracellular vesicles ■ hypertension ■ microRNAs ■ RNA, untranslated ■ sex characteristics

It is well known that excessive salt intake contributes to the development of hypertension. The deoxycorticosterone acetate (DOCA)-salt model is a preclinical model of salt-dependent hypertension in which excessive salt consumption leads to an initial increase in vascular resistance followed by elevated sympathetic outflow, similar to that seen in resistant hypertension.[1,2] Endothelin, vasopressin, and Ang II (angiotensin II) are also important contributors to the development and maintenance of elevated blood pressure (BP) in this model. While this model is associated with low renin plasma levels, activation of the brain renin-angiotensin

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NOVELTY AND RELEVANCE

What Is New?

First comprehensive identification of sex hormone–sensitive exosome-derived miRNA and protein signatures in deoxycorticosterone acetate-salt hypertension, revealing distinct androgen- and estrogen-regulated regulatory axes.

Discovery of novel sex-specific microRNA-target interactions, including miR-125b-5p/ACE2 (angiotensin-converting enzyme 2), miR-1-3p/G6PD (glucose-6-phosphate dehydrogenase), miR-143/145/MMP9 (Matrix metalloproteinase-9), miR-410-3p/AT_1R (angiotensin II type 1 receptor), and miR-378a-5p/IRAP (Insulin-regulated aminopeptidase), which influence renin-angiotensin system activity and cardiovascular remodeling.

Integration of exosomes proteomics and microRNA profiling to reveal concordant molecular pathways driving sex differences in hypertensive pathology.

What Is Relevant?

This study highlights the pivotal role of sex hormones in modulating exosomes' cargo, contributing to divergent central and peripheral molecular responses to hypertension.

This study demonstrates that specific microRNAs and their targets act as critical regulators of compensatory and pressor arms of the renin-angiotensin system, influencing blood pressure control, cardiac function, and survival.

This study provides mechanistic insight into how androgen- and estrogen-sensitive pathways contribute to male vulnerability and female protection in salt-sensitive hypertension.

Clinical/Pathophysiological Implications?

Identification of sex-specific exosome-derived molecular signatures offers novel biomarkers for early detection, prognosis, and monitoring of hypertension progression.

Targeting specific microRNA-protein regulatory axes may enable the development of precision, sex-tailored therapies for resistant or salt-sensitive hypertension.

Findings suggest potential therapeutic benefit in modulating androgen- or estrogen-regulated microRNAs to restore renin-angiotensin system balance, improve cardiovascular outcomes, and reduce sex disparities in hypertensive disease.

Nonstandard Abbreviations and Acronyms

ACE	angiotensin-converting enzyme
AGT	angiotensinogen
Ang II	angiotensin II
ANPEP	aminopeptidase N
AT_1R	angiotensin II type 1 receptor
BP	blood pressure
CSF	cerebrospinal fluid
DOCA	deoxycorticosterone acetate
EV	extracellular vesicle
MDM2	murine double minute 2
PRKCA	protein kinase C alpha
RAS	renin-angiotensin system

system (RAS) contributes to the development and maintenance of DOCA-salt hypertension as these effects are attenuated by central administration of RAS blockers.[1,3] In addition, sex hormones have been shown to affect the development of salt-dependent hypertension. While males usually show higher BP and differential expression of RAS components,[4] females have been reported to be at increased risk.[5] Recent work also reported that DOCA-salt hypertension influences the hypothalamic expression of various genes, thereby affecting the phenotype of brain cells, including neurons and microglia.[6] However, information about the mechanisms contributing to altered gene expression in DOCA-salt hypertension is lacking.

Extracellular vesicles (EVs) are phospholipid bilayer-enclosed vesicles that carry a diverse range of molecular components, such as proteins, lipids, and different classes of noncoding RNA, including microRNA. These molecules are actively secreted into the extracellular environment and facilitate intercellular communication, making them potential biomarkers for the early detection of various diseases, including hypertension.[7] In pathological conditions, alterations in both the quantity and composition of EV cargo, particularly proteins and microRNA, can reflect changes in cellular physiology. microRNAs, which are small single-stranded noncoding RNAs ranging from 19 to 23 nucleotides, regulate gene expression by binding to target mRNA, leading to their degradation or inhibition of translation. Therefore, profiling of plasma proteins and microRNA could serve to identify target genes and enriched pathways in physiological and pathological states.[8]

In this study, we aimed to reevaluate the DOCA-salt model in a sex-specific manner and analyze the microRNA content of EV isolated from the central nervous system

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and plasma. Subsequently, we examined the proteomic profiles within this hypertensive model to explore potential interactions between key proteins and microRNA, with a particular focus on sex differences to uncover potential underlying mechanisms of salt-sensitive hypertension. We observed sex differences in the expression of microRNA in BP and cardiometabolic risk factors in a common salt-sensitive hypertension model and propose that microRNA could be key targets of sex hormones in hypertension. Therefore, understanding the mechanistic effects of sex-specific microRNAs in gene pathways related to the pathophysiology of hypertension could lead toward precise cardiometabolic interventions.

METHODS

Data Availability

The authors declare that all supporting data are available within this article and in the Supplemental Material. A detailed methods section is available in the Supplemental Material with reference to key publications[3,9,10] for additional technical details.

Experiments were conducted in adult C57BL/6J mice (12–16 weeks old, Jackson Laboratory, Bar Harbor, ME) from both sexes according to ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. Mice were housed in a temperature (≈27 °C)-controlled and environmental humidity–controlled (30%–60%) facility, under a 12-hour light/dark cycle, and provided with a phytoestrogen-free standard chow (Envigo, iOS Teklad Extruded Rodent Diet 2019S, Huntingdon, United Kingdom) and water ad libitum. All procedures were approved by the Louisiana State University Health Sciences Center (3873) and the Southeast Veterans Healthcare System (620) Institutional Animal Care and Use Committees in accordance with the Principles of Laboratory Animal Care by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23, revised 1996).

Statistical Analysis

EV-microRNA isolation and proteomic analysis were processed by Creative Biolabs, Inc (Project ID: CBLK033123-1c-E), following a standardized workflow in which raw reads were aligned to miRBase, piRNAbank, and Rfam. Unique molecular identifier counts were then obtained for each microRNA and normalized by converting unique molecular identifier values to counts per million using the edgeR package, followed by $\log_2$ transformation, with minimal adjustments applied to zero-count values to enable log transformation (Figure S1). For proteomic analysis, samples were processed using LC-MS in the data-dependent acquisition mode, and protein abundances were quantified following standard BCA (Bicinchoninic acid assay)-based protein normalization and peptide processing workflows. R language and RStudio (https://rstudio.com/) were utilized as an integrated development environment, while Python and Visual Studio Code (https://code.visualstudio.com) were used as additional integrated development environments for RNA-seq data analysis. In silico analyses were performed using multiple testing correction to control false positives. Both the microRNA and proteomics data sets were analyzed using the Benjamini-Hochberg false discovery rate method. Data other than in silico were examined for normality using the Kolmogorov-Smirnov test and for homogeneity of variances using the Levene test followed by parametric or nonparametric tests, as appropriate. Data are presented as the mean±SEM. All analyses were conducted using GraphPad Prism 10 (GraphPad Software, San Diego, CA).

RESULTS

Male Mice Exhibit Exacerbated Hypertension, Cardiac Diastolic Dysfunction, and Mortality in the DOCA-Salt Model

In this first cohort, BP was recorded weekly before and after DOCA-salt initiation (Figure 1A). The 24-hour BP recording traces and averaged data from both active and resting phases (Figure 1B and 1C) show that despite a trend for females to have lower systolic BP during the active phase (125.8±4.6 versus 130.2±1.7 mm Hg, respectively), baseline systolic BP was not different between sexes, likely due to a higher heart rate in females (Figure S2A). Hypertension was established within the first week of DOCA-salt treatment and remained stable throughout the 3 weeks post-initiation. DOCA-salt treatment increased BP in both sexes, with higher systolic BP observed in males compared with females (active phase: 174.5±0.9 versus 162.2±4.2 mm Hg; $P<0.05$) over the 3-week treatment. Echocardiography data collected at the end of the third week (Figure 1D) show that ejection fraction was not changed in either male or female mice following DOCA-salt treatment. However, pulse wave and tissue Doppler showed that E/E', reflecting ventricular filling pressure and diastolic function, was increased in both sexes compared with baseline (males: 33.4±4 versus 26.6±1.9; females: 30.3±5.0 versus 26.4±4), with a greater increase in males (25.3% versus 14.4%). In addition, hemodynamics revealed increased left ventricular end-diastolic pressure (11.1±1.9 versus 4.5±0.5 mm Hg; $P<0.01$) and relaxation constant tau (6.7±1.0 versus 4.9±0.5 ms; $P<0.01$) in DOCA-salt–treated males compared with sham controls, with no changes observed in females. These data suggest worsened cardiac diastolic function in males compared with females in DOCA-salt hypertension. In addition, despite similar cardiac and renal weight increases (Table S3) among sexes, enhanced mortality (≈20%) was observed in males (Figure S2B), confirming a more severe phenotype.

DOCA-Salt Modifies the Exosome microRNA Profile in a Sex-Dependent Manner

Our analysis of microRNA expression in cerebrospinal fluid (CSF) exosomes (Figure 2A through 2C) reveals

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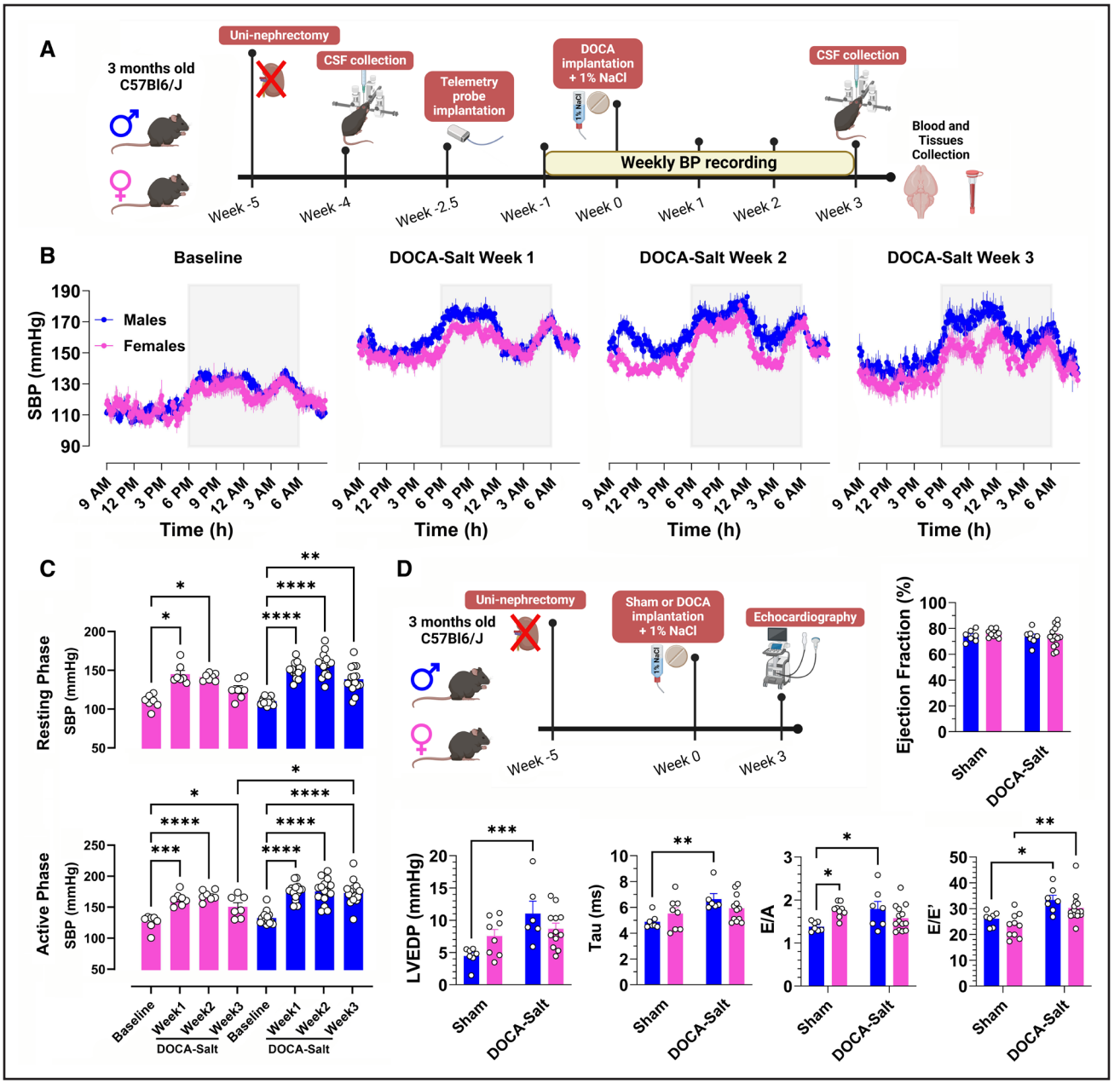

Figure 1. Hypertension and cardiac diastolic dysfunction in the deoxycorticosterone acetate (DOCA)-salt mouse model. **A**, Schematic timeline of the experimental protocol in male and female C57BL/6J mice. **B**, Representative 24-hour systolic blood pressure (SBP) recordings from baseline and weekly time points following DOCA-salt treatment, highlighting sex differences in blood pressure (BP) response (blue: males; pink: females). Quantitative analysis of SBP during resting and active phases across baseline and DOCA-salt treatment weeks. Both sexes exhibited elevated BP, with males showing significantly higher values. Data are presented as mean±SEM. Statistical significance is indicated (*P<0.05; **P<0.01; ***P<0.001; and ****P<0.0001; **C**). **D**, Experimental design for cardiac function assessment using echocardiography and invasive hemodynamics in sham- and DOCA-salt–treated mice at week 3. DOCA-salt–treated mice showed impaired diastolic function, particularly in males. Data represent mean±SEM with statistical significance noted as above. CSF indicates cerebrospinal fluid; and LVEDP, left ventricular end-diastolic pressure.

distinct expression profiles in male mice before and after DOCA-salt treatment (male DOCA-salt versus male baseline; t-statistic: 2.438; P=0.0149). In addition, we observed a difference in microRNA expression between DOCA-salt males and DOCA-salt females (male DOCA-salt versus female DOCA-salt; t-statistic: 2.807; P=0.0051), while only minor differences were detected between DOCA-salt females and baseline (female baseline versus female DOCA-salt; t-statistic: 0.918; P=0.359). In contrast, plasma samples (Figure 2D through 2F) demonstrated a significant difference in microRNA expression among all groups (male baseline versus male DOCA-salt: t-statistic: 2.446; P=0.0145; male DOCA-salt versus female DOCA-salt:

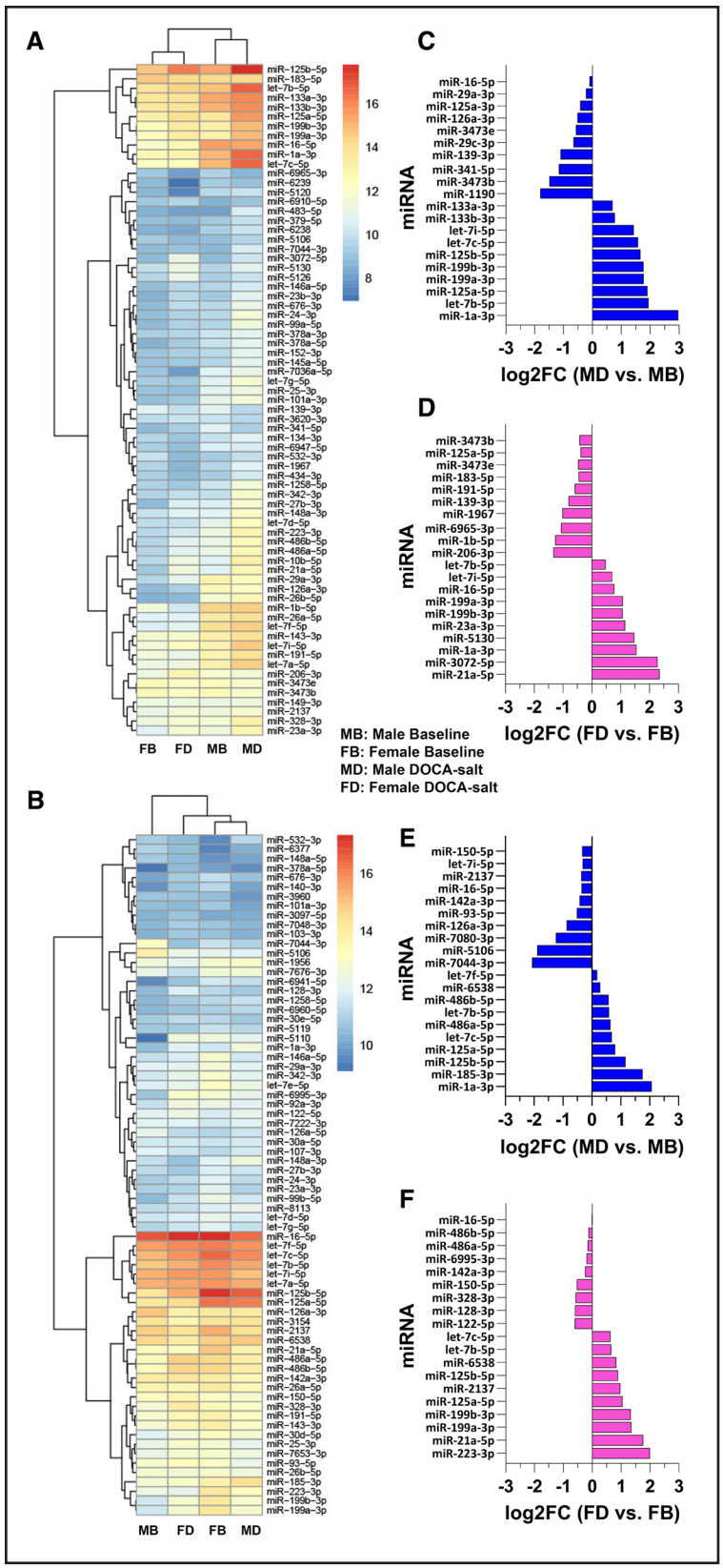

Figure 2. Deoxycorticosterone acetate (DOCA)-salt modifies the exosome microRNA (miRNA) profile in a sex-dependent manner.
A, pHeatmap miRNA analysis of cerebrospinal fluid (CSF). Top 10 miRNAs with the highest and lowest expression levels in CSF in hypertensive males (**B**) and hypertensive female mice (**C**). **D**, Heatmap miRNA analysis of plasma. Top 10 miRNAs with the highest and lowest expression levels in plasma in hypertensive males (**E**) and hypertensive female mice (**F**) treated with DOCA-salt.

t-statistic: 3.530; P=0.000425), including between females before and after DOCA-salt (t-statistic: 4.421; P<0.0001). The analysis (Gene Expression Omnibus [GEO] accession number: GSE302573) identified ≈56 microRNAs that exhibited significantly downregulated expression levels in the CSF of DOCA-salt–treated

males compared with their DOCA-salt–treated female counterparts (q-value≤0.05). Interestingly, 206 microRNAs showed significantly downregulated expression levels between DOCA-salt–treated males and DOCA-salt–treated females based on a fold-change threshold >2 (q-value≤0.05). The differential expression profiles observed in both plasma- and CSF-derived exosomes during DOCA-salt treatment across sexes suggest that microRNAs may influence the response of neuronal networks and peripheral systems involved in BP regulation, thus playing a pivotal role in DOCA-salt hypertension. In CSF samples, miR (microRNA)-1a-3p, a muscle-specific microRNA also present in the brain,[11] exhibited the highest expression level in both sexes (Figure 2C and 2D). This microRNA is cotranscribed with miR-206-3p as part of a bicistronic gene, and its expression is regulated by transcription factors such as MEF2 (Myocyte enhancer factor-2), underscoring the pivotal role of transcriptional regulation in controlling key microRNAs in the brain.[12] Furthermore, we observed that miR-125b-5p exhibited the highest expression in both sexes, while the lowest expression levels were attributed to different microRNAs: miR-16-5p in males and miR-328-3p in females in plasma samples (Figure 2E and 2F).

Compensatory RAS Members Are Targeted by microRNA in DOCA-Salt Hypertension

To validate these microRNA changes during hypertension, hypothalamic tissue and plasma samples were collected from 4-month-old C57BL/6J males and females treated with DOCA-salt (n=6–10 per group) for subsequent RNA extraction, along with their respective sham controls. We selected 10 microRNAs that might target ≥1 key molecule within the RAS pathway and associated proteins (Figure 3A and 3B), including miR-1a-3p, miR-29b-5p, miR-125b-5p, miR-133b-3p, miR-143-3p, miR-145a-5p, miR-200c-3p, miR-378a-5p, miR-410-3p, and miR-532-3p, to be evaluated in the hypothalamus and plasma (Figure 3C and 3D).

All those microRNAs had the same expression level in males and females, with the exception of miR-200c-3p, which was elevated in the hypothalamus of females, possibly playing a key role in regulating ACE (angiotensin-converting enzyme) 2 expression in baseline conditions. Interestingly, this microRNA was upregulated in the plasma of hypertensive males, suggesting a potential compensatory mechanism, albeit insufficient to curb the development of hypertension. Our results highlight a significant increase in miR-1a-3p, miR-29b-5p, and miR-125b-5p expression in DOCA-salt males in both the hypothalamus and plasma (Figure 3C and 3D). This is important as these microRNAs are thought to target components of the compensatory RASs (ACE2, AT_2R, APN [Aminopeptidase N], APA [Aminopeptidase A], and ATRAP [Angiotensin II type-1 receptor–associated protein]) that regulate Ang II signaling. On the other hand, miR-133b-3p and miR-200c-3p, which target the PRR ((Pro)renin receptor) and ACE2, respectively, were only increased in the plasma (Figure 3D). Interestingly, miR-145a-5p that is thought to regulate ADAM17 (A disintegrin and metalloproteinase 17) and ACE and miR-410-3p targeting AT_1R (Ang II type 1 receptor) and MDM2 (murine double minute 2) were significantly downregulated in the plasma of DOCA-salt males, potentially contributing to the hypertensive phenotype (Figure 3D). In contrast, in hypertensive males and females, the compensatory RAS could have benefited from the downregulation of miR-378a-5p, which limits the expression of AT_2R, IRAP (insulin-regulated aminopeptidase), and APA. Of note, miR-125b-5p was the most upregulated in hypertensive males and females' plasma, matching the in silico data (Figure 2E and 2F). Together, these data show that DOCA-salt hypertension is associated with an upregulation of microRNA in the hypothalamus and plasma that tend to favor overactivity of the RAS and Ang II signaling.

Sex Hormones Alter the Development of Hypertension and Associated Phenotypes

To investigate the potential mechanisms associated with sex-dependent phenotypes in DOCA-salt hypertension, we performed additional experiments in gonadectomized mice. Contrary to the first cohort of mice, baseline systolic BP was significantly lower in sham females compared with sham males (active phase: 123.5±1.6 versus 132.4±1.7 mm Hg; $P<0.05$; Figure 4A). Gonadectomy did not affect baseline BP in females (active phase: 126.1±2.5 mm Hg), but it was significantly reduced in gonadectomy males (118.0±0.7 mm Hg; $P<0.05$). DOCA-salt hypertension developed faster in sham males (Figure S3A) and remained higher compared with their female counterparts following 3 weeks of treatment (active phase: 175.5±1.5 versus 160.3±3.8 mm Hg; $P<0.05$; Figure 4A). Interestingly, in female mice, although baseline systolic BP was not different between groups, DOCA-salt treatment produced a significantly higher response in gonadectomy mice, notably in the second week (active phase: 177.3±3.3 mm Hg; $P<0.05$ versus sham) where it reached a similar level as in sham males (175.5±1.5 mm Hg). On the other hand, in males, gonadectomy resulted in a blunted increase in systolic BP following DOCA-salt, as seen in the third week (active phase: 158.4±2.4 mm Hg; $P<0.05$ versus sham) where it reached a similar level as in sham females (160.3±3.8 mm Hg). Our results show that gonadectomy had modest and time-dependent effects on systolic BP during the development of

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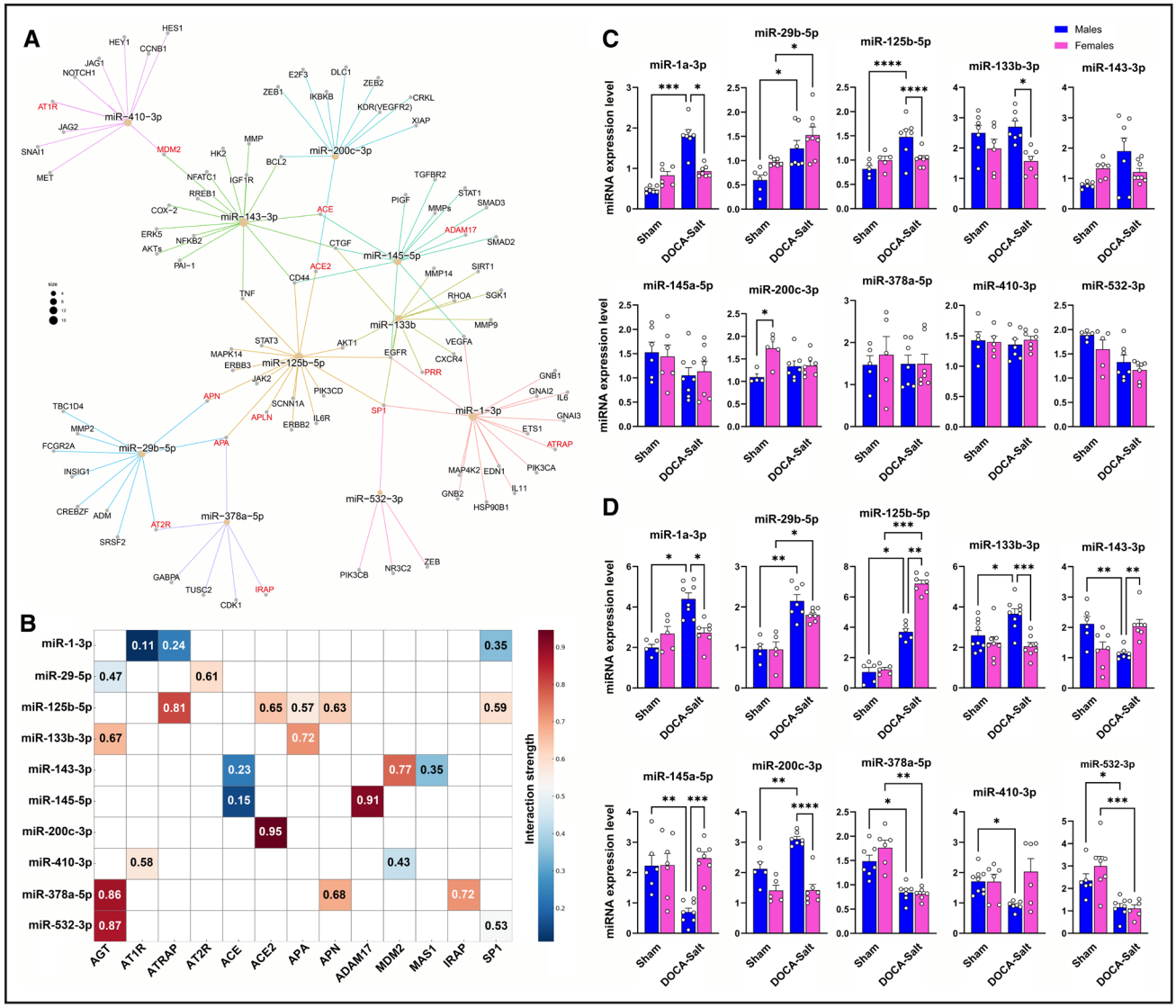

Figure 3. Compensatory renin-angiotensin system (RAS) members are targeted by microRNA (miRNA) in deoxycorticosterone acetate (DOCA)-salt hypertension.

A, MiRNA profiles and targets based on cNETmap analysis. **B**, pHeatmap of miRNA-target interaction scores based on miRTargetLink 2.0 based on the Context++ score percentile in the human/mouse. Hypothalamus (**C**) and plasma (**D**) samples from male and female C57BL/6J mice treated with DOCA-salt (n=6 per group), along with their respective sham controls, were collected for validation of miRNA expression.

hypertension, with ovariectomy transiently enhancing the hypertensive response in females and orchiectomy slightly attenuating the response in males at later time points on DOCA-salt–induced hypertension. Ejection fraction and E/E' were not altered by gonadectomy in either DOCA-salt–treated males or females (Figure S3B). However, the survival rate (Figure S3C) was significantly impaired by gonadectomy in DOCA-salt females (81.2% versus 100%), while a modest improvement was observed in DOCA-salt males (80% versus 84.2%). In addition, the increased weight of the kidney, a major androgen-sensitive tissue, in DOCA-salt hypertension was blunted (≈14%) in gonadectomy males (Figure S3D; Table S3; $P<0.001$) but not in gonadectomy females. No changes in heart weight were observed following gonadectomy in either DOCA-salt males or females (Figure S3D; Table S3).

Sex Hormones Regulate the Expression Profiles of microRNA

The upregulation of miR-1a-3p and miR-125b-5p levels, which occurred in the hypothalamus and plasma during hypertension, was prevented in castrated males (Figure 4B and 4C). While hypothalamic levels of miR-133b-3p and miR-200c-3p were not affected, plasma levels were also reduced by castration, suggesting that testosterone contributes to the upregulation of these

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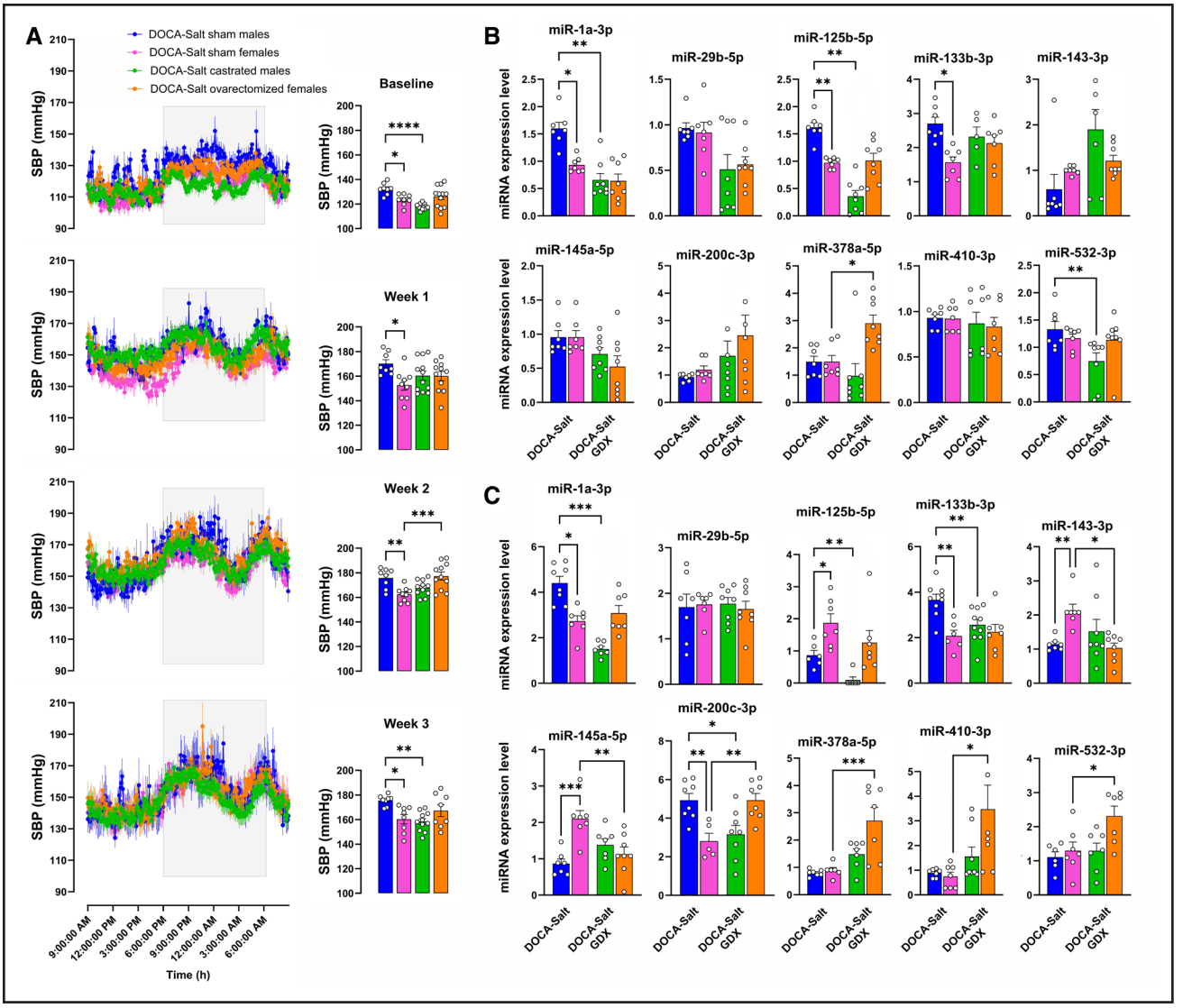

Figure 4. Impact of sex hormones on blood pressure regulation and microRNA (miRNA) expression in deoxycorticosterone acetate (DOCA)-salt hypertensive mice.

Systolic blood pressure (SBP) measurements across baseline and 3 consecutive weeks in DOCA-salt–treated male (blue), female (pink), castrated male (green), and ovariectomized female (orange) mice. **A**, SBP is plotted over a 24-hour period, with corresponding bar graphs summarizing mean SBP for each group. Quantitative expression levels of selected miRNAs, comparing sex-specific and hormone-depleted DOCA-salt mouse models in the hypothalamus (**B**) and plasma (**C**). Statistical significance: **P*<0.05, ***P*<0.01, and ****P*<0.001.

microRNAs in DOCA-salt hypertension. Among those, miR-125b-5p emerged as strongly upregulated by testosterone and repressed in its absence, highlighting the importance of sex hormones on microRNA regulating key components of the RAS, notably the compensatory RAS. On the other hand, ovariectomy was associated with a plasma downregulation of miR-143-3p and miR-145a-5p, while hypothalamic levels seemed unaffected, supporting an inhibitory effect of ovarian hormones on these microRNAs. Ovariectomy also facilitated the upregulation of other microRNAs such as miR-378-5p and miR-200c-3p in both plasma and hypothalamus, while miR-532-3p and miR-410-3p were only increased in plasma. Together, our data indicate that sex hormones play a key role in regulating the expression of several microRNAs in the hypothalamus and periphery during DOCA-salt hypertension.

Protein Cargo of EV in DOCA-Salt Mice

Protein analysis of plasma EV revealed distinct expression profiles in DOCA-salt male and female mice compared with their sham controls (Figure 5A). A total of 1780 protein-coding genes were identified in both sexes (GEO accession number: GSE302573). Among these, 276 proteins exhibited significant downregulated, and 93 were upregulated, with a q-value of <0.05 between male DOCA-salt mice compared with the female DOCA-salt

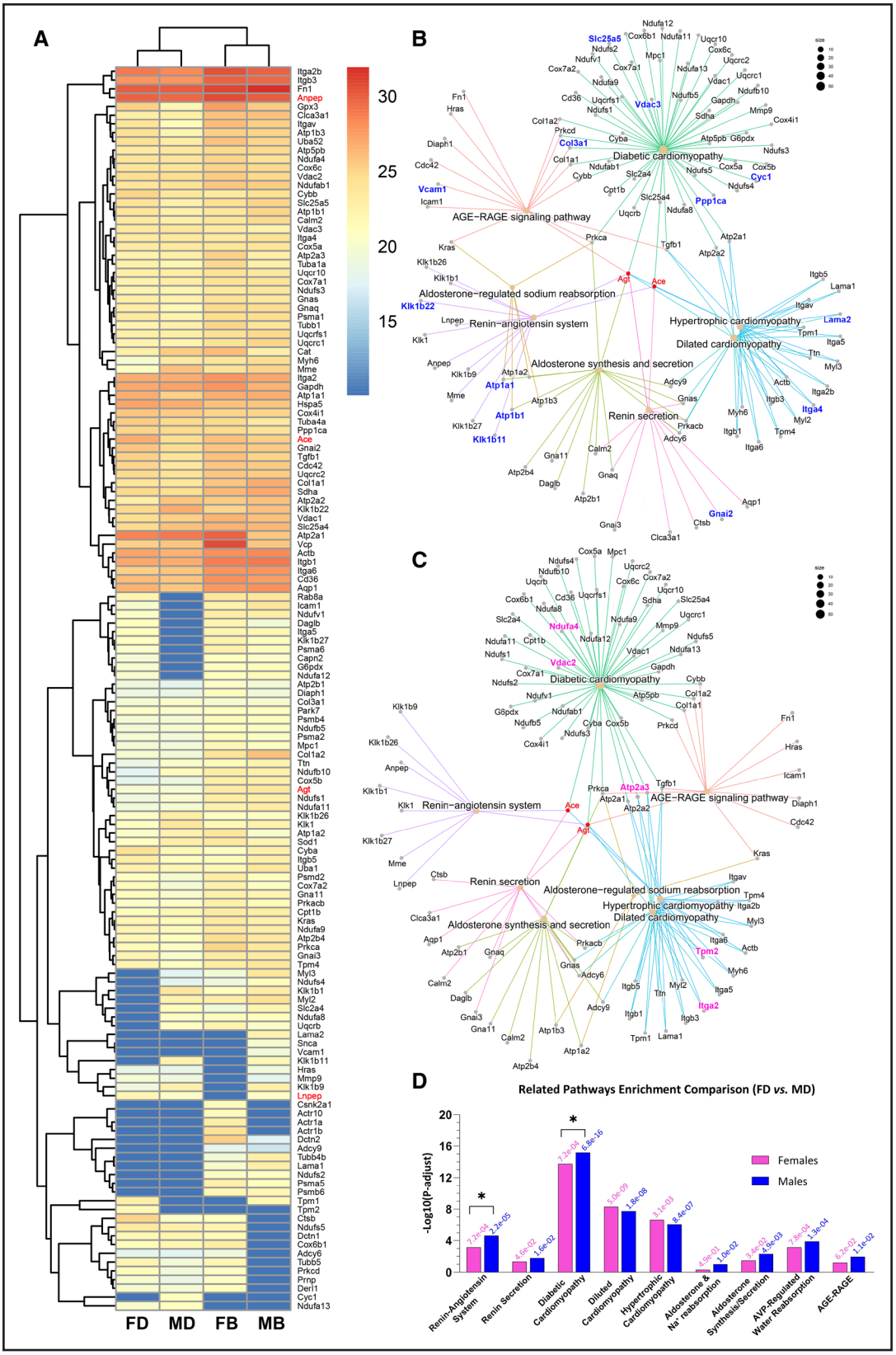

Figure 5. Proteomic profiling of extracellular vesicle (EV) cargo from the plasma of deoxycorticosterone acetate (DOCA)-salt hypertensive mice.

A, Heatmap showing the differential protein expression in EV from male (male DOCA-salt [MD] vs male baseline [MB]) and female (female DOCA-salt [FD] vs female baseline [FB]) DOCA-salt mice compared with sex-matched baseline (pretreatment). **B**, cNetplot illustrating Kyoto Encyclopedia of Genes and Genomes (KEGG)-enriched pathways in MD EV proteome, highlighting key renin-angiotensin (*Continued*)

mice. In addition, the ingenuity pathway analysis identified 257 altered pathways in male mice, of which 17 pathways were specific to this sex. Among the 235 independent proteins identified, 132 demonstrated differential expression in DOCA-salt hypertensive males compared with DOCA-salt hypertensive females. Furthermore, 72 proteins were exclusively detected in hypertensive male mice. Figure 5A presents a pHeatmap illustrating the expression profile of genes involved in the RAS and associated pathways. We then enriched known pathways related to the RAS using their respective IDs and descriptions. Enrichment statistics were assessed in terms of *P* values, adjusted *P* values, and q-values. Notably, diabetic cardiomyopathy ranked among the top 10 signaling pathways based on pathway enrichment scores (Figure 5B and 5C). In addition, our analysis indicates that the RAS is significantly overexpressed in males compared with females, with q-values of 1.4×10^{-5} and 5.0×10^{-4}, respectively. We also identified sex-specific protein profiles through cNETmap analysis. To visualize the pathways according to enrichment scores derived from our data, we generated a cNETmap that highlights hypertension-related pathways (Figure 5B and 5C). Our findings reveal that AGT (angiotensinogen) and ACE are involved in all the analyzed pathways. In addition, PRKCA (protein kinase C alpha) is uniquely shared between the aldosterone synthesis and secretion and diabetic cardiomyopathy pathways. PRKCA has been shown to enhance calcium sensitivity in vascular smooth muscle cells, promoting vasoconstriction.[13] It also functions as a downstream effector in the Ang II signaling pathway via AT_1R. These combined actions suggest that, alongside key regulators including AGT and ACE, PRKCA plays a pivotal role in the pathophysiology of hypertension associated with diabetes and obesity. Furthermore, the interaction of mediator genes such as the *Atp2a* family appears to be stronger between diabetic cardiomyopathy and other related pathways in hypertensive male mice compared with females. This suggests that male hypertensive mice exhibit stronger activation of gene cascades associated with hypertension compared with females. Consistent with our observation of impaired cardiac function, 21 genes in males and 22 genes in females were commonly involved in both hypertrophic cardiomyopathy and dilated cardiomyopathy in response to DOCA-salt treatment. Among the 106 genes in males and 98 genes in females, associated with the 8 selected pathways, including the RAS and related signaling cascades, 14 genes were unique to males (Figure 5B), and 6 were unique to females (Figure 5C). Most importantly, our proteomic analysis revealed a significant upregulation of 2 key pathways including RAS and diabetic cardiomyopathy in male DOCA-salt mice compared with female DOCA-salt mice, highlighting the influence of sex hormones on key proteins overlapping in these pathways (Figure 5D).

Evaluation of the Concordance Between EV-Derived microRNAs and Proteins

Among the 106 genes implicated in the selected pathways, including the RAS, our in silico analysis using miRTarBase-derived data from miRTarLink2 (https://ccb-compute.cs.uni-saarland.de/mirtargetlink2/)[14] identified 20 genes as targets of our selected panel of 10 microRNAs. Their expression levels were assessed using EV-plasma and CSF RNA-seq data (Figure 6A), and the expression profiles of 16 of these genes were further evaluated in silico based on our proteomics data (Figure 6B), revealing that *G6pdx* (glucose-6-phosphate dehydrogenase) exhibited the most significant differential expression between sexes. In silico analysis suggests that miR-1-3p may play a pivotal role in regulating *G6pdx* mRNA, leading to its downregulation and potentially contributing to the development of cardiac hypertrophy or fibrosis. Our data highlighted an upregulation of miR-1-3p in hypertensive male mice, accompanied by a marked downregulation of *G6pdx* expression, suggesting an inverse correlation between these 2 molecules under the influence of androgens. Furthermore, our results demonstrated a significant increase in MMP9 (Matrix metalloproteinase-9) expression in DOCA-salt–treated females compared with males. While early MMP9 activation may support vascular compliance and BP regulation, sustained elevation can promote hypertension and vascular injury.[15] A strong inverse correlation between plasma miR-143/145 levels and serum MMP9 was reported in intracranial aneurysms, often associated with hypertension.[16] Given that estrogens are known to upregulate miR-143/145 expression,[17,18] these findings suggest a potential regulatory axis involving ERα (Estrogen receptor alpha)/miR-143/145/MMP9. Further investigation into this axis may clarify its role in BP control. Among the genes susceptible to being regulated by our microRNAs, 4 were identified to be directly involved in the RAS (Figure 6C). While AGT did not exhibit significant sex-specific differences in expression between DOCA-salt–treated male and female mice, ACE, the key enzyme responsible for converting Ang I to the active peptide Ang II, exhibited differential expression between DOCA-salt–treated males and females. This sex-dependent dysregulation of ACE supports enhanced Ang II production, a critical factor in the initiation and maintenance of hypertension. These findings align with evidence indicating activation of brain RAS components

Figure 5 Continued. system (RAS)–related pathways. **C**, cNetplot of KEGG-enriched pathways in FD EV proteome, showing distinct sex-based enrichment in RAS-associated pathways. **D**, Comparison of pathway enrichment (–log10 adjusted *P* value) between male and female DOCA-salt groups (FD vs MD) across major RAS-related pathways. Asterisks indicate significant differences.

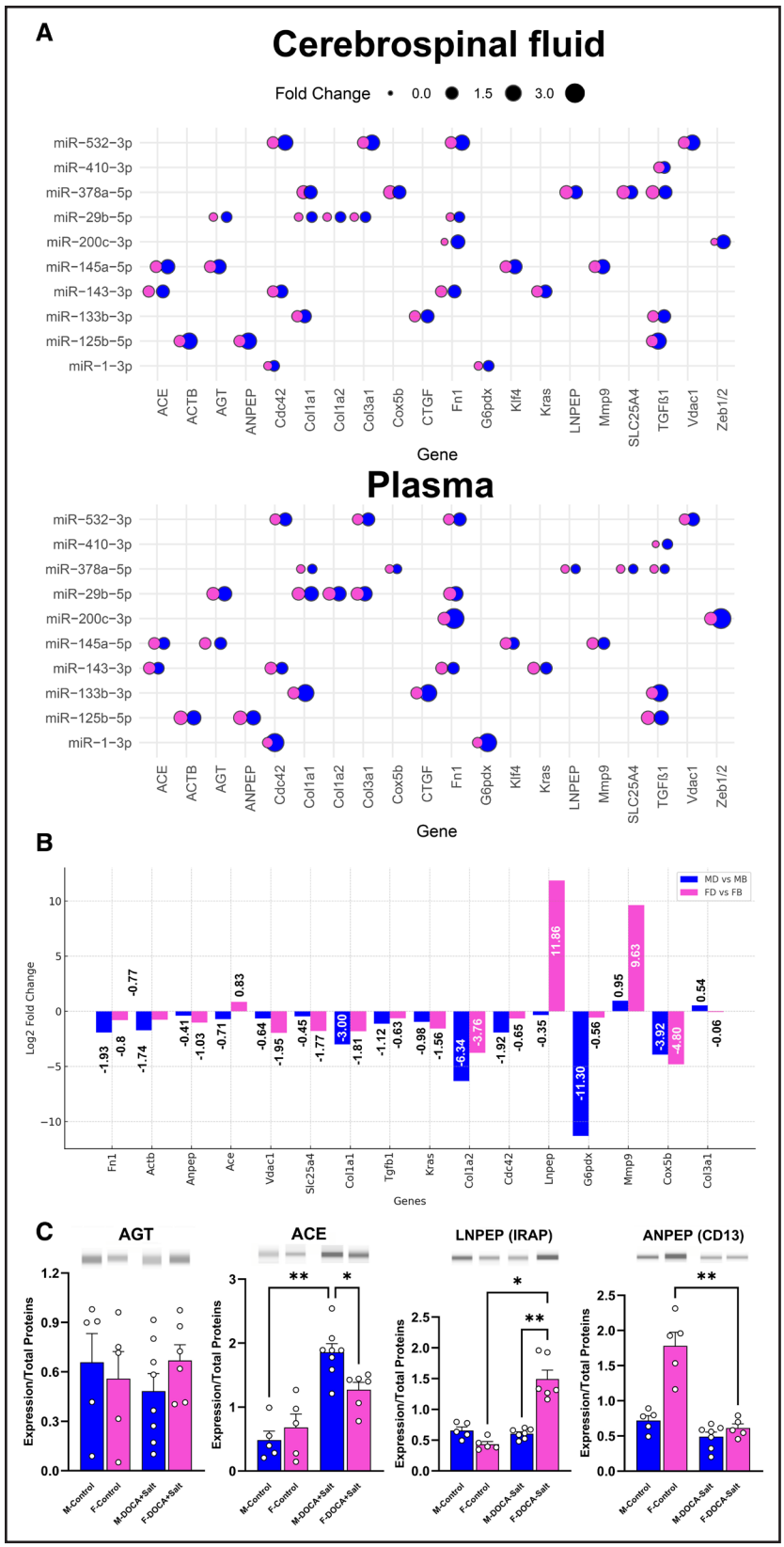

Figure 6. Sex-specific profiling of extracellular vesicle (EV)–associated proteins targeted by microRNAs (miRNAs) in hypothalamus and plasma.
A, Bubble plots depicting predicted interactions between a panel of EV-derived miRNAs and their target proteins in cerebrospinal fluid (CSF; **top**) and plasma (**bottom**). Bubble size indicates fold change; color represents sex (pink: female; blue: male). **B**, Bar plot showing log2-fold changes in plasma EV protein expression between hypertensive and control groups in males (male DOCA-salt [MD] vs male baseline [MB], blue) and females (female DOCA-salt [FD] vs female baseline [FB], pink), based on in silico analysis. **C**, Quantification of key renin-angiotensin system (RAS)–related proteins (AGT [angiotensinogen], ACE [angiotensin-converting enzyme 2], LNPEP [Leucyl/cystinyl aminopeptidase (gene encoding IRAP)] [IRAP (insulin-regulated aminopeptidase)], and ANPEP [aminopeptidase N; CD13]) in the plasma via capillary Western across male and female control and deoxycorticosterone acetate (DOCA)-salt groups. Statistical significance is indicated (*P<0.05; **P<0.01).

under DOCA-salt–induced hypertensive conditions, particularly in males.[3,19] Our analysis revealed a significant upregulation of IRAP (LNPEP [Leucyl/cystinyl aminopeptidase (gene encoding IRAP)]), but not of ANPEP (aminopeptidase N/CD13), which is involved in the formation of Ang IV, an inhibitor of IRAP activity. Notably, a negative correlation was observed between miR-378a-5p and IRAP expression, suggesting a potential

regulatory interaction influenced by estrogen signaling. In addition, miR-125b-5p was predicted to target multiple genes, including ANPEP, ACTB (Beta-actin), and TGFβ1 (Transforming growth factor beta-1), which are central to various metabolic and vascular pathways implicated in hypertension regulation (Table S4). These findings highlight the intricate microRNA-mediated control over RAS-related components and metabolic regulators, particularly under sex hormones' influence.

DISCUSSION

Sex differences are well established in the development of hypertension and other cardiovascular diseases, with premenopausal women being relatively protected. Over the past 2 decades, extensive research has underscored the potential of noncoding RNA, particularly microRNA, as innovative therapeutic agents in a wide range of diseases. These microRNAs hold substantial potential in managing chronic conditions such as hypertension by modulating key regulatory networks at the posttranscriptional level. However, there is a lack of information about the role of sex hormones on microRNA expression in hypertension, including commonly used experimental models, such as DOCA-salt hypertension.

In this study, we first confirmed that sex hormones mediated cardiovascular phenotypes in this model. Consistent with early work in DOCA-salt rats,[20] we observed exacerbated BP increases in males compared with females in mice. In addition, we observed, for the first time, a worsening of cardiac diastolic dysfunction in males. As reported by Mathieu et al,[21] we observed mortality in males, likely due to the more severe cardiovascular phenotype including worsened diastolic dysfunction and hypertension, but not in females following DOCA-salt treatment. However, the exact cause of death for these mice was undetermined. The mechanisms involved in the sex dimorphism in the DOCA-salt model remain largely unknown. Previous publications highlighted sex-specific responses to immune,[22] endothelial,[23] RAS,[24] and autonomic[21,25] systems in DOCA-salt hypertension and associated cardiovascular phenotypes, highlighting that sex hormones could play important roles in these responses. In our study, hypertension and mortality were exacerbated by ovariectomy and attenuated by orchiectomy in DOCA-salt mice, suggesting a protective role of estrogen and a detrimental role of androgen in the development of hypertension. These data are consistent with previous observations in DOCA-salt rats,[26] showing that testosterone treatment in gonadectomized males restored the development of hypertension to levels similar to those observed in intact DOCA-salt males, while estradiol treatment in gonadectomized females reduced hypertension to levels similar to DOCA-salt females, further confirming the contribution of sex hormones in the development of DOCA-salt hypertension.

It is well established that the brain RAS plays a critical role in the development of DOCA-salt hypertension. However, little is known of sex-specific changes in the brain RAS that could contribute to hypertension in this model. In rats, Dai et al[24] showed that intracerebroventricular infusion of the AT_2R antagonist, PD123319, promoted DOCA-salt hypertension in females but not in males. In mice, Mathieu et al[21] surprisingly observed that selective ablation of the prorenin receptor in the RVLM (Rostral ventrolateral medulla) resulted in a further increase in systolic BP in females but not in males, which was paradoxically associated with upregulation of compensatory RAS components. These data suggest sex-dependent changes in brain RAS in the DOCA-salt model; however, it is unknown how sex hormones contribute to these sex-specific phenotypes. To address this question, we investigated EV-associated microRNA profiles in the DOCA-salt model, with particular emphasis on sex-specific alterations in both central and peripheral compartments. Our findings reveal distinct male and female expression of microRNA, within the hypothalamus, a key target in neurogenic hypertension,[1] and plasma, which we propose as the main targets for sex hormones to critically influence hypertension-related molecular pathways.

Notably, our findings reveal a group of androgen-sensitive microRNAs including miR-1a-3p, miR-133b, and miR-125b-5p. Our data show that miR-1a-3p was significantly upregulated in male DOCA-salt mice in both the hypothalamus and plasma, and this effect was reversed in gonadectomy males, indicating androgen-mediated regulation. To the best of our knowledge, this is the first report of miR-1a-3p upregulation in a hypertensive model and is consistent with a previous study, showing that the androgen receptor directly binds to the regulatory region of miR-1, thus enhancing its expression.[27] In addition, miR-133b-3p, though unchanged in the hypothalamus, was elevated in the plasma of male DOCA-salt mice and reduced after gonadectomy. This pattern, along with prior evidence of androgen receptor activation of the miR-133b promoter, reinforces its androgen sensitivity.[28,29] Although known as muscle-specific microRNA, these microRNAs can be expressed in nonmuscle tissues, including the brain, where they may influence neuronal differentiation and synaptic plasticity.[11] Interestingly, miR-125b-5p expression was altered in both sexes and compartments, with greater hypothalamic elevation in hypertensive males and higher plasma levels in hypertensive females. Its androgen sensitivity was further supported by reduced levels in gonadectomy males. In silico analysis identified key RAS-related targets of this microRNA, including ATRAP, SP1 (Specificity protein 1), ACE2, APA, and APN. Previous studies have also linked miR-125b-5p to hyperglycemia,[30] stroke,[31] and cardiac fibrosis,[32] supporting its broader cardiovascular relevance.

On the other hand, we observed another group of estrogen-sensitive microRNAs including miR-200c-3p, miR-143/145, cluster miR-378a-5p, and miR-532-3p. MiR-200c-3p was upregulated in plasma but not in the hypothalamus, suggesting a peripheral origin. ACE2, a pivotal component of the compensatory RAS, is one of its predicted targets. Given the estrogens' known inhibitory effects on miR-200c-3p, our findings could suggest a sex hormone-regulated mechanism potentially contributing to hypertension via ACE2 suppression.

Furthermore, miR-145-5p and miR-143-3p (the miR-143/145 cluster) were reduced in the plasma of male DOCA-salt mice and further downregulated in estrogen-deficient gonadectomy females. Interestingly, miR-145-5p targets ADAM17, a key player in ACE2 downregulation.[3,19] Our analysis also points to MDM2 and ADAM17 as targets of miR-143-3p and miR-145-5p, respectively. Estrogens seem to upregulate this cluster, supporting its role in vascular protection.[33] MiR-378a-5p was slightly downregulated in hypertensive mice, with increased expression following ovariectomy, indicating an inverse regulation by estrogens. This microRNA is encoded within the *PPARGC1B* gene, which coactivates ERα, supporting an estrogen-related regulation.[34] In addition, miR-532-3p was unchanged in the hypothalamus but significantly downregulated in the plasma of hypertensive females and ovariectomized mice, again pointing to estrogen-driven regulation.[35] Moreover, miR-410-3p, a well-known neuroprotective microRNA, showed a modest plasma decrease in hypertensive males. Its function appears to be estrogen-regulated in a context-specific manner.[36] Not surprisingly, our analysis identified AT_1R as a key target, supporting its therapeutic relevance in hypertension. Moreover, our findings demonstrate that several microRNAs, including miR-125b-5p, possess diverse and sometimes opposing roles in hypertension depending on tissue context and hormonal status. For example, SP1, a miR-125b-5p target,[37] can either enhance AT_1R transcription[38] or act on ACE2,[39] depending on hormonal cues. Estrogens have also been shown to modulate SP1 activity, adding another layer of complexity.

Based on our EV-derived proteomics analysis, we identified 16 genes involved in 8 critical pathways related to the RAS. These genes are predicted to be targeted by ≥1 of the 10 proposed microRNAs (Table S4). Among these, *G6pdx* showed the most significant sex-dependent differential expression (Figure 6B). Per the miRTargetLink 2.0 database, *G6pdx* is predicted to be a strong target of miR-1-3p,[40,41] opposing hypertension by regulating ROS and maintaining endothelial nitric oxide signaling.[42,43] These findings imply a potential inverse correlation between miR-1-3p and *G6pdx* under the regulatory influence of androgen hormones and an ERα-miR-143/145-MMP9 regulatory axis that may influence sex-specific BP control and vascular remodeling in hypertension. In addition, our results indicate that ACE expression is regulated in a sex-dependent manner, potentially under androgenic control. This regulation may lead to enhanced production of Ang II, a key effector in the initiation and progression of hypertension. These findings are consistent with prior studies demonstrating activation of RAS components in DOCA-salt–induced hypertensive models, particularly in males. Interestingly, we observed an upregulation of IRAP in hypertensive females. Beyond its involvement in the RAS, IRAP also cleaves oxytocin and vasopressin, which play key roles in DOCA-salt hypertension, and, therefore, may exert additional RAS-independent regulatory effects in hypertension, particularly in females, some of which could involve the 14-3-3 protein pathway.[44] These insights support IRAP as a potential therapeutic target for sex-specific hypertension. Our in silico results suggest that miR-378a-5p can be a regulator of IRAP, and both our in silico predictions and ex vivo validations demonstrate a probable inverse correlation between miR-378a-5p and IRAP expression. This regulatory axis appears to be influenced by estrogen levels, aligning with previous reports linking elevated miR-378a-5p to altered cellular processes in estrogen-sensitive contexts.[34]

Our study highlights several potential regulatory axes including miR-125b-5p/ACE2, miR-1-3p/G6PD, miR-143/145/MMP9, miR-29-5p/AT_2R, miR-410-3p/AT_1R, and miR-378a-5p/IRAP, which may play critical roles in the maintenance and progression of hypertension. The predicted interactions warrant validation using rigorous molecular approaches to establish causal relationships. Crucially, confirmation of these regulatory interactions in human samples is essential to guide the development of novel therapeutic strategies for hypertension. While gonadectomy modifies the hormonal milieu in a manner consistent with reduced androgenic signaling in males and reduced estrogenic signaling in females, which may, in turn, influence the regulation of hypertension and microRNA expression, sexual dimorphism in DOCA-salt mice could also be independent of BP.[45] The definitive causal attribution to individual hormones requires additional experiments-such as selective hormone replacement after gonadectomy, cross-sex hormone administration, pharmacological antagonism, or inhibition of aromatase activity, which are essential next steps for future studies aimed at dissecting the precise contributions of individual sex hormones. In attempting to link microRNA and protein expression, one should also be mindful of the origin of exosomes, notably in the plasma, which could be secreted by tissues with different susceptibilities to hypertension.

In summary, our findings highlight sex-specific differences in microRNA profiles associated with hypertension, which offers a roadmap for further investigation into new mechanisms aimed at developing sex-tailored therapeutic strategies. Through comprehensive analysis of EV-derived microRNAs and proteins at the in silico, in

vitro, and ex vivo levels in a hypertensive model, we reveal novel insights into the coordinated interplay between key microRNAs and proteins within the RAS. These interactions contribute to our understanding of the molecular mechanisms underlying hypertension and open new avenues for personalized treatment approaches.

By elucidating the intricate connections between sex hormones and microRNA-mediated regulation, targeted therapeutic strategies can be further refined in chronic hypertension. In particular, the miR-125b-5p/ACE2, miR-1-3p/G6PD, miR-143/145/MMP9, miR-410-3p/AT_1R, and miR-378a-5p/IRAP regulatory axes emerge as promising targets for intervention, with potential relevance for treating chronic hypertension, especially in young men and postmenopausal women.

PERSPECTIVES

This study provides a comprehensive description of the microRNA and protein changes associated with the DOCA-salt hypertension model in the CSF and plasma of intact and gonadectomy mice. We propose several models to be validated in future studies for the potential role of sex hormones on microRNA and gene expression, notably with regard to components of the RAS. Validation of these regulatory pathways could provide new avenues to investigate sex-dependent personalized therapies in hypertension.

ARTICLE INFORMATION

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Author Contributions

V. Tarhriz, H. Xia, and E. Lazartigues designed experiments. V. Tarhriz, H. Xia, A. Patel, A. Scarborough, K. Miao, H.N. Mir, and L. Abkhooie performed the experiments. M. Eivazi and V. Tarhriz performed in silico data analysis. F. Mauvais-Jarvis contributed to the sex differences analysis. V. Tarhriz, H. Xia, and E. Lazartigues wrote the manuscript. All authors contributed to manuscript revision. All authors read and approved the final manuscript.

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Disclosures

None.

Supplemental Material

Supplemental Material and Methods
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