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Reprograming of transcriptional profile of colonic organoids from patients with high blood pressure by minocycline

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Abstract

Minocycline, an anti-inflammatory antibiotic drug, rebalances impaired gut microbiota, attenuates neuroinflammation and lowers high blood pressure in animal models of hypertension and in hypertensive patients. Our objective in this study was to investigate if antihypertensive effects of minocycline involve the expression of gut epithelial genes relevant to blood pressure homeostasis using human colonic 3-dimensional organoid culture and high-throughput RNA sequencing. The data demonstrates that minocycline could restore impaired expression of functional genes linked to viral and bacterial immunity, inflammation, protein trafficking and autophagy in human hypertensive organoids.

Keywords

Hypertension; Minocycline; Colonic organoid; Transcriptome; Drug-gene interaction

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CRediT authorship contribution statement

Conceptualization Ideas: MKR, CJR, JL; Methodology: JL, EMR; Validation: JL; Formal analysis: JL, MKR, EMR; Investigation: JL, EMR, MKR, EMH, SMS, EA, CEF; Writing - Original Draft: JL, MKR; Writing - Review & Editing: JL, EMR, MKR, EMH, SMS, EA, CEF; Supervision: MKR; Project administration: EMR; Funding acquisition: MKR, CJR.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

Neuroinflammation and gut microbial dysbiosis are emerging hallmark pathophysiological events in the development of systemic hypertension (HTN). Evidence for this concept is primarily derived from comprehensive experimentation in animal models that link an impaired gut-brain axis with HTN [1]. For example, antihypertensive angiotensin-converting enzyme inhibitors rebalance gut microbiota, attenuate microglia activation and neuroinflammation [1]. Furthermore, minocycline, a tetracycline derivative distinguished by possession of both anti-inflammatory and antibiotic properties and freely permeable through the blood brain barrier, attenuates high blood pressure (BP), decreases neuroinflammation and rebalances gut microbiota in animal models of HTN [1]. This antihypertensive effect of minocycline has been validated in our open label pilot study where it reduced BP and decreased activated microglia and systemic blood $CD4^+$ cells harboring gut inflammatory markers in high-risk treatment resistant hypertensive patients [1,2]. It is important to better understand the antihypertensive mechanism of minocycline because of its therapeutic potential so we have planned a randomized, double-blinded, parallel-design trial. Therefore, our objective in the current study was to test the hypothesis that antihypertensive effects of minocycline involve influencing expression of gut epithelial genes relevant to BP control using human colonic 3-dimensional (3D) organoid culture and high-throughput RNA sequencing.

2. Materials and methods

2.1. Human colonic 3D organoid culture

The University of Florida Institutional Review Board approved the protocol for this human study (no. 201903360). We received the consent of 16 subjects with high systolic blood pressure (SBP) (156 ± 4 mmHg, high blood pressure, HBP) and 22 subjects with normal BP (110 ± 3 mmHg, normal blood pressure, RBP) for the study. Details of clinical characteristics of these subjects were described in our previous publication [3]. Within 30 min of the collection of biopsies by colonoscopy, human colonic crypts were isolated from the biopsies (~2 × 4 mm) of colon descending aspects in these subjects with gentle cell dissociation reagents (STEMCELL Technologies) including 2 mM EDTA for 90 min at 4 °C. The crypts were grown and maintained as 3D-spheroid cultures in Matrigel (BD Biosciences) containing organoid growth medium (STEMCELL Technologies) with recombinant human Noggin [Pepro-Tech] and EGF [BioLegend], recombinant human IGF-1, FGF-basic [FGF-2; BioLegend] and R-spondin1 [R&D], Y-27632 [STEMCELL Technologies], and A83-01 [Tocris], as described previously [3]. The human colonic organoids were cultured for 8 days, then treated with 1 mM minocycline for 24 h in the following experiments.

2.2. RNA-seq

Total RNA was extracted from human colonic organoids with RNeasy Plus Mini Kit (Qiagen) showing RNA integrity numbers between 7.1 and 9. cDNA was generated using a SMART-Seq HT kit (Takara Bio) and RNA Seq libraries were prepared with Nextera

DNA Flex Library Prep kit and Nextera DNA Unique Dual Indexes Set A (Illumina) and sequenced on NovaSeq6000 instrument (Illumina, at 2 × 150 bp, aiming for 50 million paired-end reads per sample) at the University of Florida NextGen DNA Sequencing Core Facility. RNA seq analysis was performed using a CLC genomics workbench (Qiagen 21.0.4). Data were normalized to reads per kilobase of transcript per million mapped reads (RPKM). False Discovery Rate (FDR)-corrected p value <0.05 was used as the criterion for significance. iDEP V0.94 was used for gene set enrichment analysis (GSEA) by the pre-ranked fgsea method [4]. Heat maps of differentially expressed genes (DEGs) in enriched pathways were built using supervised hierarchical clustering in Heatmapper [5].

2.3. Identification of Hub genes

The protein-protein interaction network of these DEGs was constructed by the online tool STRING (https://string-db.org) with a combined score >0.4. Subsequently, CytoHubba analysis was performed to mine the hub genes using the Closeness algorithm in CytoScape [6]. A range from white to red color (lowest to highest, respectively) indicates molecular complex detection (MCODE) score. MCODE score of 2 was set as a threshold.

3. Results and discussion

Characteristics of subjects for colon biopsies and establishment of three-dimensional organoids from normal (RBP) and high BP (HBP) subjects are presented in our previous publication [3]. Organoids from both groups were comparable and minocycline treatment showed no differential effects on cell morphology and numbers; nevertheless, minocycline had significant effects on the transcriptomic program. It upregulated 2470 genes and downregulated 2586 genes in RBP organoids, while 2231and 2548 genes were up- and downregulated in HBP organoids, respectively (Fig. 1A). Interestingly, more than half of the differentially expressed genes (n = 2834 DEGs) overlapped between RBP and HBP in the minocycline-treated group (Fig. 1B), indicating that minocycline caused the differential transcriptomic profiles of RBP and HBP colonic organoids. Further analysis of all DEGs by gene set enrichment analysis (GSEA) showed that gene sets increased in HBP organoids such as SRP-dependent translational, protein targeting to membranes, viral genes, nuclear transcribed mRNA catabolic process genes and nonsense-mediated decay process in HBP vs. RBP [3], were downregulated by minocycline (Fig. 1C). Correspondingly, decreased expression of gene sets in HBP vs. RBP for cell-substrate junction assembly and immune responses including $CD4^+$ alpha-beta cells activation and interferon gamma-mediated signaling were increased in minocycline-treated HBP organoids (Fig. 1C). Heatmap analysis further demonstrated that the top 20 DEGs majorly belonged to the gene sets for organic cyclic compound biosynthesis (e.g., *BMP4*, *NME1*, *PCNA* and *TESC*) altered by minocycline, canrenone and captopril both in RBP and HBP organoids (Fig. 1D). Notably, some of the top 20 DEGs may be associated with the development and pathophysiology of HTN (Fig. 1D). For example, BMP4, encoded for bone morphogenic protein-4 inducing HTN in intact animals by increasing superoxide production and being linked to vascular pathology in hypertensive patients [7], was significantly downregulated by minocycline both in RBP and HBP organoids (Fig. 1D).

It is noteworthy to point out that these minocycline treatment-modulated gene sets have been implicated in gut barrier leakiness and gut pathology in HTN. Importantly, the pathogenic expression of 155 DEGs of RBP vs. HBP were normalized in HBP organoids and 64 DEGs in RBP organoids by minocycline (Fig. 2A and Table 1). A notable example is *TNFRSF11A*, encoding tumor necrosis factor receptor superfamily member 11a that is linked to HTN [8] and critical in vascular calcification, was significantly upregulated in HBP organoids compared to RBP organoids but significantly downregulated by minocycline in both RBP and HBP organoids (Fig. 2A and Table 1). These minocycline responsive genes in HBP organoids have roles in immune responses (*OASL*, *SQSTM1*, *PARP14*, *CXCR3*), protein targeting and localization (*RAB39B*, *ATG4A*) and epithelial cell differentiation (*ALDC*, *SPRED3*) as indicated by GSEA. Interestingly, some of these genes are also influenced in RBP by minocycline. Next, we employed the Drug Gene International Database (dgidb.org) to identify potential drug targets such as *OASL* (2′−5′-oligoadenylate synthetase like), *SQSTM1* (Sequestosome 1) and *AK4* (Adenylate kinase 4), whose expression was altered by minocycline in HBP organoids (Fig. 2A).

Finally, we constructed the protein-protein interaction (PPI) network of altered DEGs and mined hub genes using CytoScope software. Fig. 2B and C shows that SQSTM1 in HBP vs. HBP with minocycline, is the most dense and relevant hub. This gene is associated with protein scaffolding, is involved in cellular processing, autophagy, metabolic programing and is linked with neurodegenerative diseases, obesity and atherosclerosis [18]. SQSTM1 has a high degree of association with proteins such as ISG15, OASL, PARP4, RAB39B, TNFR3F11A, ATG4A and ZFYVE1, which are generally linked to bacterial and innate immunity, T cell function, antiviral activity, inflammation, and autophagy and implicated in HTN. Responsiveness of SQSTM1 in RBP and HBP organoids suggests that minocycline exerts a moderating effect on existing pathways rather than exerting a unique influence.

In conclusion, our observations, for the first time, demonstrate that minocycline (i) exerts direct effects on gut epithelia and (ii) rebalances genes whose expression is altered in HBP. Most of these genes are relevant in viral and bacterial immunity, inflammation, protein trafficking and autophagy. Thus, we suggest that the antihypertensive effects of minocycline are, in part, due to reprograming of gut epithelial transcriptome. Whether these minocycline-induced transcriptomic changes are the primary event that moderate gut dysbiosis and neuroinflammation or minocycline independently affects these processes to ameliorate HTN remains to be investigated.

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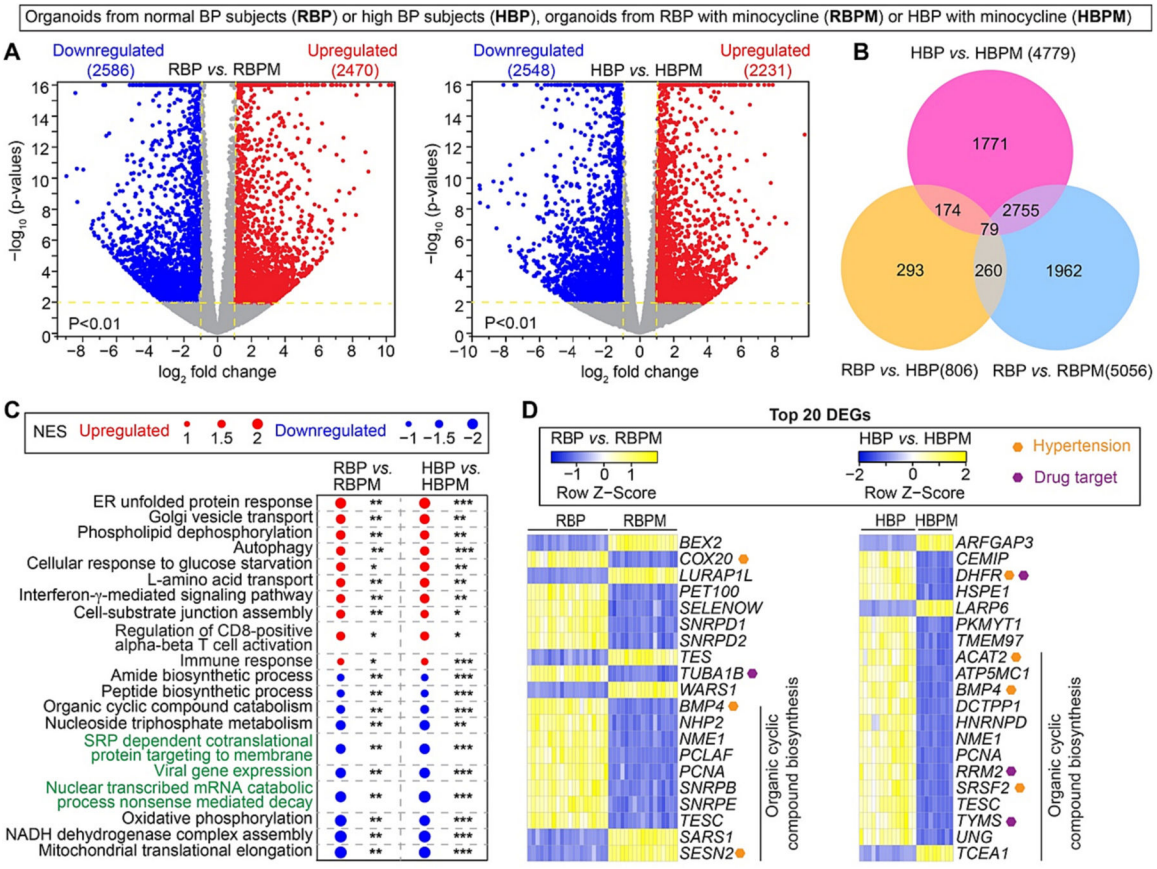

Fig. 1.

Effects of minocycline on transcriptomic profiles in colonic organoids from subjects with normal (RBP) and high (HBP) blood pressure. (A) Volcano plot showing the numbers of differentially expressed genes (DEGs) in organoids from RBP and HBP treated by 1 mM minocycline (M). $p < 0.01$, absolute fold change ≥2. (B) Venn diagram showing the numbers of the overlapping DEGs between RBP and HBP treated by minocycline (M). (C) Hallmark pathways significantly enriched in the organoids from RBP and HBP treated with minocycline (M). Not significant (N.S.) adjusted $p > 0.05$, * adjusted $p < 0.05$, ** adjusted $p < 0.01$, ***adjusted $p < 0.001$. Pathways highlighted with green font were impaired in HBP organoids and colonic tissue and could be repaired by minocycline treatment. NES: normalized enrichment score; ER: endoplasmic reticulum; NADH: nicotinamide adenine dinucleotide; SRP: signal-recognition particle; CD: cluster of differentiation. (D) Heat map of the top 20 DEGs in the annotated pathways of the organoids from RBP and HBP treated by minocycline treatment.

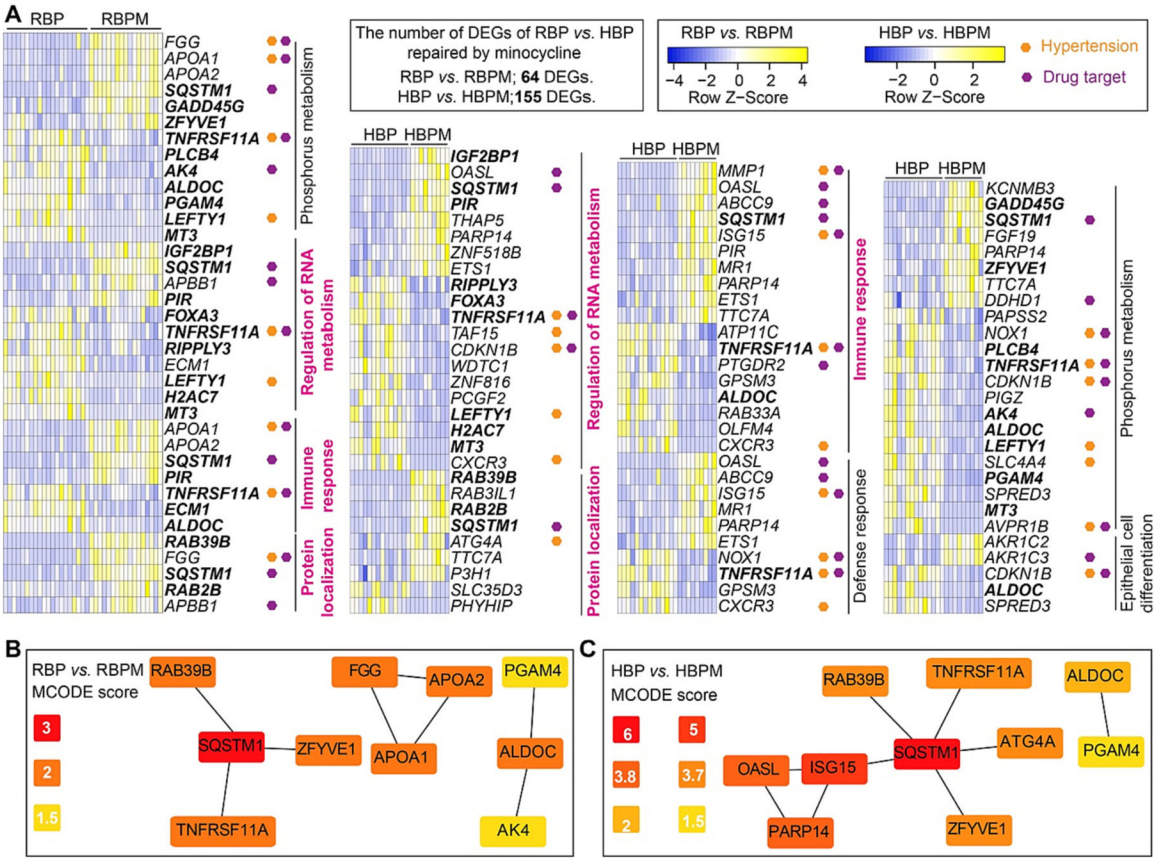

Fig. 2.

(A) Heat map of the DEGs of RBP vs. HBP repaired by minocycline in the organoids from RBP and HBP. The genes whose expression was repaired in both RBP and HBP organoids are indicated by bold font. Identification of the top 10 hub genes in the DEGs of RBP vs. HBP repaired by minocycline in the organoids from RBP and HBP. (B): RBP vs. RBPM; (C): HBP vs. HBPM.

Table 1

The HTN-associated DEGs of RBP vs. HBP repaired by minocycline in the organoids from RBP and HBP.

Gene name	Protein name	RBP vs. HBP Fold change/P value	HBP vs. HBPM Fold change/P value	DEGs associated HTN
TNFRSF11A	Tumor necrosis factor receptor superfamily, member 11a	1.61/0.03	−3.21/2.14E-6	Association of gene polymorphisms in RANKL/RANK/OPG system with hypertension and blood pressure in Chinese women [8]
CDKN1B	Cyclin-dependent kinase inhibitor 1B	1.71/0.02	−3.98/6.21E-7	Neutrophil extracellular traps accelerate vascular smooth muscle cell proliferation via Akt/CDKN1b/TK1 accompanying with the occurrence of hypertension [9]
LEFTY1	Left-right determination factor 1	2.25/0.04	−6.13/3.39E-5	Association of Maternal hypertension and diabetes with variants of the NKX2-5, LEFTY1 and LEFTY2 genes in children with congenital heart defects: a case-control study from Pakistani Population [10]
ATG4A	Autophagy related 4A cysteine peptidase	−1.81/0.03	1.85/0.02	Predisposition to cortical neurodegenerative changes in brains of hypertension prone rats [11]
MMP1	Matrix metallopeptidase 1	−3.09/3.67E-3	17.9/6.90E-19	Associations of MMP1, 3, 9 and TIMP3 genes polymorphism with isolated systolic hypertension in Chinese Han population [12]
ISG15	Interferon-induced 15 KDa protein	−2.1/1.16E-3	4.19/1.22E-11	Interferon-stimulated gene 15 pathway is a novel mediator of endothelial dysfunction and aneurysms development in angiotensin II infused mice through increased oxidative stress [13]
NOX1	NADPH oxidase 1	2.02/0.01	−2.58/3.96E-4	Nox1 is involved in angiotensin II–mediated hypertension: a study in Nox1-deficient mice [14]
SLC4A4	Sodium bicarbonate cotransporter	2.35/0.02	−7.36/3.13E-6	Identification of IGF1, SLC4A4, WWOX, and SFMBT1 as Hypertension Susceptibility Genes in Han Chinese with a Genome-Wide Gene-Based Association Study [15]
CXCR3	C-X-C motif chemokine receptor 3	4.43/4.03E3	−31.17/4.96E-6	Left ventricular dysfunction and CXCR3 ligands in hypertension: from animal experiments to a population-based pilot study [16]
AVPR1B	Arginine vasopressin receptor 1B	4.09/6.68E-3	−21.59/2.39E-3	Effects of maternal hypertension on cord blood Arginine vasopressin receptor expression [17]