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"BRCC36 Contributes to ACE2 Deubiquitination in Salt-Sensitive Hypertensive Female Mice"

According to the Centers for Disease Control and Prevention, hypertension directly contributed to 691,095 deaths in 2021, making it one of the leading causes of death in the United States. The brain renin-angiotensin system (RAS) plays a pivotal role in the pathogenesis of hypertension. Angiotensin-II (Ang-II) binds to the Ang-II type 1 receptor (AT1R) and mediates the vasoconstrictive and pro-hypertensive effects. On the other hand, angiotensin-converting enzyme 2 (ACE2) mitigates the pro-hypertensive effects of Ang-II by cleaving it into Ang-(1-7), a vasodilator. We have previously reported that Ang-II mediates the internalization and degradation of ACE2. In hypertensive males, we identified NEDD4-2 and UBR1 as E3 ubiquitin ligases targeting ACE2. Based on discovery proteomics mass spectrometry for hypothalamic tissue extracted from normal and hypertensive mice, BRCC36, a Lys-63-specific deubiquitinase, was observed to be downregulated in hypertensive mice. We further analyzed BRCC36 protein levels in the brain tissues of male and female mice. Baseline BRC336 protein levels were significantly higher in the brain of female mice (6-fold compared to baseline males, $p<0.05$). This massive increase was reversed by Ang-II treatment and was associated with the downregulation of ACE2 protein levels in the brain. Furthermore, HEK293 cells transfected with BRCC36 failed to reduce ACE2 protein levels and activity observed in non-transfected cells, supporting the relationship between these proteins. In this study, we aimed to understand the role of BRCC36 in the deubiquitination of ACE2 in hypertensive female mice. We hypothesize that infusion of BRCC36 protein in the brain of female mice will result in the deubiquitination of ACE2 thus an increase in its availability and might lower BP levels. To test our hypothesis, C57BL/6J female mice (10 weeks old, n=20) were subjected to uni-nephrectomy surgery to increase their salt sensitivity. A week later, mice were implanted with radiotelemetry probes for 24 h recording of the hemodynamic parameters (BP, HR, and activity). Baseline BP was recorded followed by subcutaneous implantation with Deoxycorticosterone Acetate (DOCA) pellets (50 mg/mouse) together with 0.9% saline water for drinking ad libitum. After 7 days, BP was recorded to ensure that mice acquired the hypertensive phenotype. Hypertension was confirmed after 1 week of DOCA-Salt treatment (Mean±SEM 136 vs. 108mmHg). Mice were then divided into two groups for intracerebroventricular infusion of either BRCC36 or artificial cerebrospinal fluid (aCSF) (50 µg/mouse, n=8/group) using a subcutaneous mini-osmotic pump connected through a catheter to an ICV cannula. BP will be recorded weekly for 2 weeks to monitor changes in blood pressure among the treatment groups. After 2 weeks mice will be euthanized, and protein will be extracted from the hypothalamus for capillary western analysis to measure ACE2 and BRCC36 protein expression among the two groups.