Extracellular Vesicles and Deiminated Proteins in the Brain of Naked Mole-Rats altered by Acute Hypoxia Exposure

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Introduction

Fig1. Naked mole-rat (*Heterochephalus glaber*)

Naked mole-rats (*Heterocephalus glaber*) are mammalian species with unusual resistance to hypoxia, adapting their metabolism by reducing energy demand and maximize the efficiency of metabolic pathways.

Extracellular Vesicles (EVs) are circulatory membrane vesicles in body fluids and play important roles in cell communication and pathological processes via transfer of EVs cargo, including modified protein cargo.

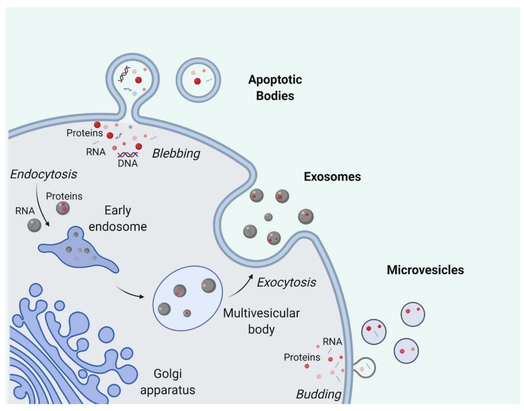


Fig2. Extracellular vesicles classification and biogenesis

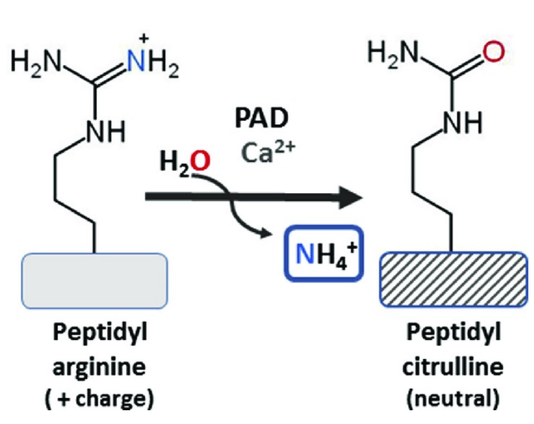


Fig3.Citrullination/Deiminitation mechanism

Citrullination/deimination is a post-translational modification that converts arginine in to citrulline caused by a family of five enzymes (PADs), associated with a range of pathological conditions.

Material and Methods

- **Animals' treatment**

Naked mole-rat were exposed to either 21% O_2 (normoxia) n=5 animals per group or 7% O_2 (hypoxia) n=5 animals per group. Plasma was extracted from whole blood and aliquots stored at -80 until analysis.

- **EVs Isolation and characterisation**

Plasma EVs isolation by **differential centrifugation,** EVs quantification by **Nanoparticle tracking analysis (NTA)**, EVs characterisation by **Western Blotting (WB)** and **Transmission electron microscopy (TEM)**

- **F95-Enrichment**

Identification of deiminated/citrullinated proteins in plasma EVs by **Immunoprecipitation** using F95 pan-citrulline antibody.

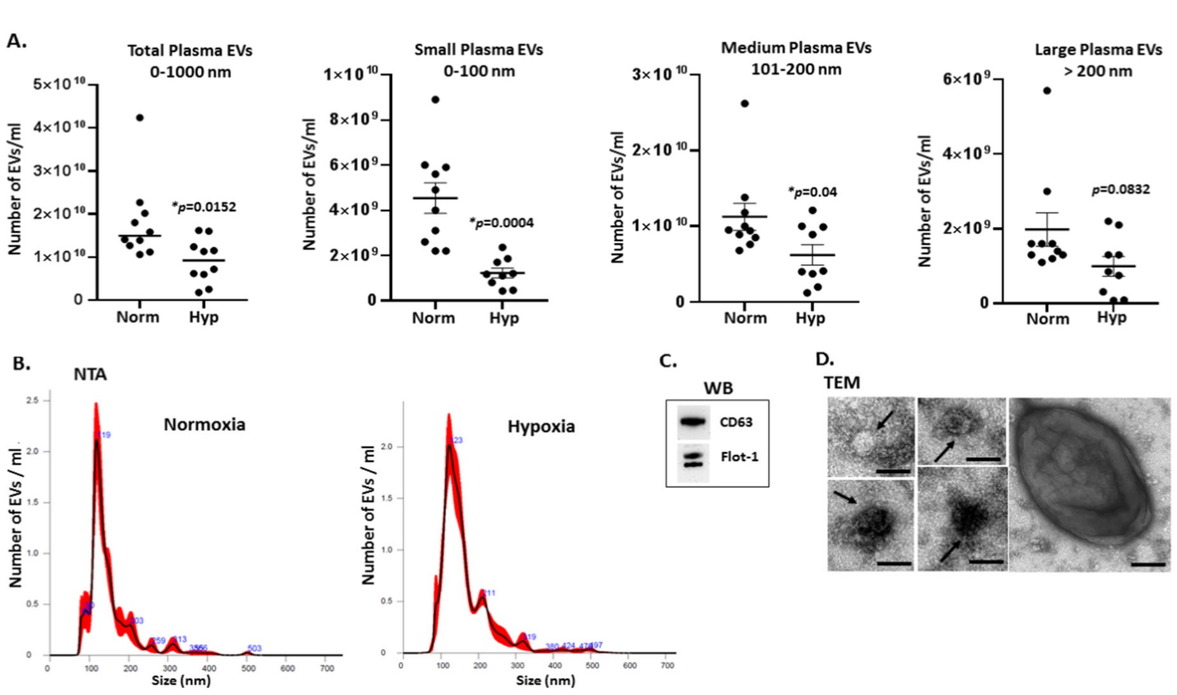
- **LC-MS/MS proteomics Analysis**

To determine whole protein content of the plasma EVs and citrullinated protein elutes from the plasma EVs. Protein Hits were assessed against the naked mole-rat protein database CCP_*Heterocephalus_glaber*_20190911 (21,449 sequences; 10,466,552 residues).

- **Protein Interaction Network Analysis**

STRING analysis was conducted to identify GO and KEGG pathways for protein from total and deiminated plasma EVs

Results



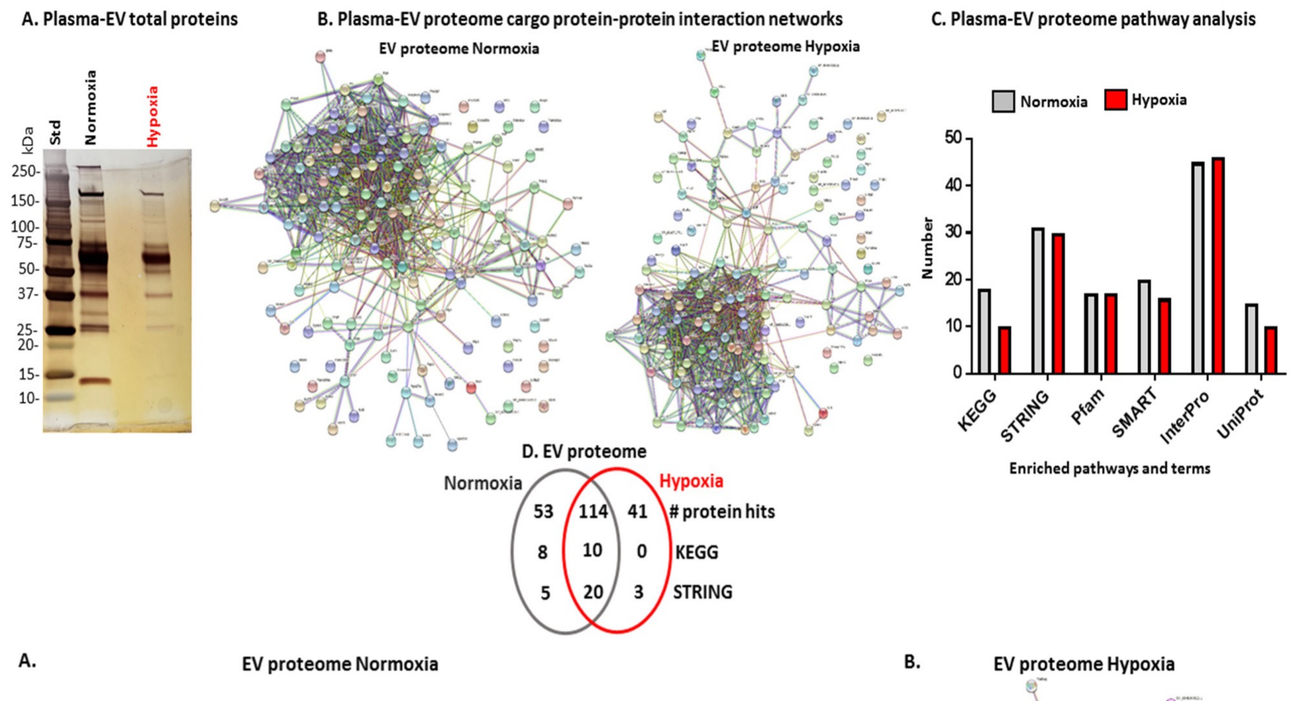


Fig4. EV profile trends from plasma of naked mole-rats treated for 4h in normoxia or hypoxia. (B)Nanoparticle tracking analysis (NTA); (C) Western Blotting; (D) Transmission electron microscopy

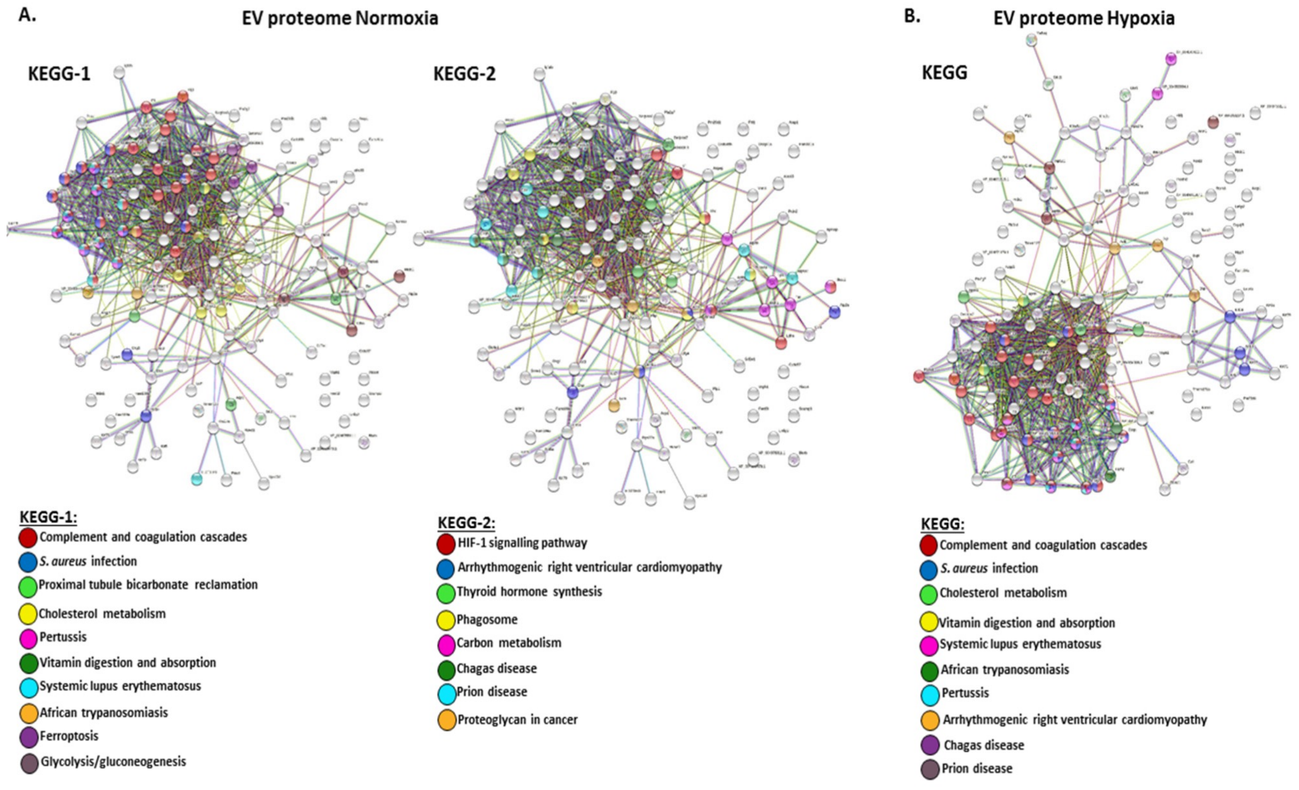


Fig5. Proteomic analysis of plasma EVs from normoxia-and hypoxia-treated naked mole-rats

Conclusion

Naked mole-rat model was used to assess CNS-related responses of PADs in hypoxic protection/tolerance and identify whether circulating EVs signatures could reveal fingerprint for whole-body hypoxia-tolerant responses ,and citrullination-specific signatures in EVs assessed in animals under normal versus hypoxic conditions. Our findings highlight modifications in circulatory EVs proteome signature, indicating a shift to re-directing resources systemically in response to acute hypoxia.

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