

Decoding the Human Adrenal Cortex Matrisome: The Composition and Regulation of ECM Proteins in the Outer and Inner Microenvironment

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Alterations in the extracellular matrix (ECM) composition induce distinct cellular responses in both normal and pathological tissues. Here we described by proteomic analysis the ECM proteins comprising the outer (OF) and inner (IF) fractions of the human adrenal cortex. The human adrenal gland comprises a cortex constituted of three zones: glomerulosa (ZG), fasciculata (ZF), and reticular (ZR), and a central medulla surrounded by a capsule (C). The OF (C+ZG) and IF(ZF+ZR) from the human adrenal cortex (n=5) obtained from USP-Capital Death Verification Service (Ethics Committee nº6.524.377) were decellularization, digested, and purified according to the protocol adapted from Kremer et al., 2024. Raw LC-MS/MS files were processed using MaxQuant 2.4.9 software/Andromeda search engine against human databases and Perseus software v2.0.11. The total identified and regulated proteins, based on LFQ intensity with a minimum of 60% valid values, were compared with the Human Matrisome (<https://doi.org/10.1074/mcp.M111.014647>, accessed in January 2024). Proteins were annotated using UniProtKB codes (<https://www.uniprot.org/>) and Gene Ontology- AmiGO2 (GO) (<https://amigo.geneontology.org/amigo/landing>). Protein-protein interaction networks and functional enrichment analysis were performed using the STRING platform (<https://string-db.org/>). A qualitative analysis identified 121 ECM proteins in the human adrenal cortex, and 106 ECM proteins were cross-validated using the Human Matrisome database. 15 proteins (APCS, APOA1, APOA4, APOE, CALR, CLU, CMA1, GLG1, HNRNPM, HSP90AA1, HSP90B1, KRT1, PKM, PSAP, SPP2) were selected for inclusion in the Human Adrenal Cortex Matrisome, based on annotations obtained from the database of GO. Through a quantitative analysis, 32 ECM-proteins were grouped into ECM-structure, ECM-component, Associated with collagen, cellular proliferation, and differentiation subgroups. 17 proteins are up-regulated (COL1A1, COL1A2, COL14A1, COL6A1, COL6A2, COL6A3, ELN, FBN1, FN1, LAMA5, LAMB2, LAMC1, PKM, ASPN, BGN, KRT1, TGFB1) and 2 (VCAN, VTN) down-regulated in OF compared to IF. The expression of COL1 and COL14 were validated through histochemistry and immunohistochemistry, respectively. In summary, we described, for the first time, a specific matrisome of the human adrenal cortex, demonstrating differences in ECM composition in the outermost zone. These differences may contribute to understanding the microenvironment of the adrenal cortex, its morphology, and its function in health and disease.

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