Proteomic Signature of the Extracellular Matrix in Adrenocortical Neoplasms

Henrique Sanchez Ortega[1], Jean Lucas Kremer[1], Talita Souza Siqueira[2], Claudia Blanes Angeli[3], Maria Candida Barisson Villares Fragoso[4], Leo Kei Iwai[5], Giuseppe Palmisano[3], Claudimara Ferini Pacicco Lotfi[1]

[1.] ICB 3 - USP, Laboratório de Estrutura e Função Celular - Departamento de Anatomia - Instituto de Ciências Biomédicas da Universidade de São Paulo, Avenida Professor Lineu Prestes 2415, São Paulo - SP;
[2.] LIM 29 - FMUSP, Laboratório de Nefrologia Celular, Genética e Molecular - LIM29 - Faculdade de Medicina da Universidade de São Paulo , Avenida Doutor Arnaldo, 455, Cerqueira César -SP;
[3.] ICB 2 - USP, Laboratório de Glicoproteômica - Departamento de Parasitologia - Instituto de Ciências Biomédicas da Universidade de São Paulo, Avenida Professor Lineu Prestes 1374, São Paulo - SP;
[4.] LIM 42 - FMUSP, Laboratório de Hormônios e Genética Molecular, LIM 42, Prédio dos Ambulatórios , Avenida Doutor Enéas Carvalho de Aguiar 255 - Cerqueira César - SP;
[5.] LETA / CeTICS, Laboratório de Toxinologia Aplicada, LETA/CeTICS, Instituto Butantan, Avenida Vital Brasil 1500, São Paulo - SP;

The extracellular matrix (ECM) comprises macromolecules that form a complex three-dimensional network, filling the intercellular spaces and maintaining tissue structure and function. The ECM regulates essential cellular processes, including adhesion, differentiation, and cell signaling[1, 2]. In rat and human normal adrenal glands, composed of a cortex and medulla surrounded by a capsule[3], the ECM was described by Kremer et al. (2024) ?' ?. In addition, the impact of the ECM on proliferation and steroidogenesis was described in the normal and tumor adrenal cortex?[-1]?. This study aims to provide insights into the composition and regulation of the ECM in the human adrenal cortex neoplasm microenvironment. The ECM composition was compared to adult human normal adrenal cortex and adrenocortical neoplasms. We described by proteomic analysis the ECM protein signatures of the human normal adrenal (HNA, n=5) obtained from the USP-Capital Death Verification Service and neoplasm fragments of Primary Macronodular Adrenal Hyperplasia (PMAH) with (w) ARMC5 mutation (n= 5) and without (wt) mutation (n= 5); Adrenal Cortical Adenoma (ACA, n=8); Adrenal Cortical Carcinoma (ACC, n=8) and Oncocytic Adrenal Carcinoma (OACC, n=7) obtained from the Adrenal Unit, Laboratory of Hormones and Molecular Genetics LIM/42, FMUSP (Ethics Committee nº 6.524.377). The sample preparation for proteomic analysis was performed as described by Kremer et al. (2024) ?, without samples decellularization. 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. 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/). In total, 362 ECM-proteins were qualitatively identified and validated in the Human Matrisome DB 2.0 (https://doi.org/10.1074/mcp.M111.014647, accessed in May 2024). Of the total ECM-proteins, 175 were quantitatively analyzed and categorized into 59 glycoproteins, 18 collagens, 54 ECM regulators, 8 proteoglycans, 22 ECM-affiliated proteins, and 14 secreted factors. Statistical comparison between groups showed 19 proteins differentially expressed in HNA vs. PMAH w; 12 proteins HNA vs. PMAH wt; 15 proteins HNA vs. ACA; 25 proteins HNA vs. ACC; 26 proteins HNA vs. OACC; 9 proteins PMAH w vs. PMAH wt; 26 proteins ACA vs. ACC; 15 proteins ACC vs. OACC. The findings will be validated through database analysis and histochemical and immunohistochemical approaches. Supported by Fapesp.

Agradecimentos: Jean Lucas Kremer, Talita Souza-Siqueira, Claudia Blanes Angeli, Maria Candida B V Fragoso, Leo Kei Iwai, Giuseppe Palmisano, Claudimara Ferini Pacicco Lotfi