Proteomic Analysis of Murine Renal Cell Carcinoma and Implications of Sleep Restriction in Its Development.

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INTRODUCTION: Along with cancer, another big epidemic that modern society is exposed to is the lack of sleep. The problem won a spot after COVID-19 pandemic, but for the general population, quality of sleep was already a privilege. Considering advances in protein analysis tools, it's important to assess the implications of such habit on the development of disorders like cancer, highlighting possible predictive biomarkers.

METHODS: With this purpose we conducted a 21 day experiment that aimed to evaluate changes in tumoral protein expression related to sleep restriction in twelve BALB/c mice inoculated with a renal cell carcinoma (RENCA) suspension. Half were assigned as controls, maintaining a regular sleep cycle. The others underwent a sleep restriction protocol. From 4 pm to 10 am, restricted mice stayed in cages filled with 2cm of water and plaster-based platforms, where they could freely move and eat. This arrangement significantly reduced their quality of sleep, because as soon as they got to the paradoxical stage, their muscles would lose strength, making them fall in the water and wake up. On the 21st day, the animals were euthanized and portions of their upper legs were removed for further analysis. The samples were stored in formalin solution until being included in paraffin and the blocks were used for microscope slides confection, histopathological investigation and later for proteome analysis. In addition, in order to establish the RENCA cell proteome, culture samples were submitted to the FASP (Filter Aided Sample Preparation) protocol. Protein identification was carried out by a liquid-chromatography coupled to mass spectrometry system (LC-MS). The spectral data acquisitions were performed using DDA mode with selection of the 10 most abundant ions for sequencing. Raw data was processed using MaxQuant software.

RESULTS: Initially, the goal was to search for the most relevant groups of proteins in RENCA cultures. Protein quantity visualization and basic analysis was conducted with Perseus software, assessing range and reproducibility. 650 hits were returned after data treatment, with a good overall distribution, as verified by histograms, scatter plots and profile plots. In order to highlight the most significant biological processes taking place, classification was performed using KEGG, according to protein intensities. Then, ShinyGO 0.8, a graphical tool, was used in a complementary analysis to generate a summarized representation of these pathways, being the most important categories: proteasome, ribosome, spliceosome, Aminoacyl-tRNA biosynthesis and Citrate cycle. Finally, Reactome provided a wide overview of the biochemical processes.

The proteomic analysis of tumor biopsies derived from control and sleep-restricted animals is still in progress. However, it was already possible to evaluate the microscope slides regarding the histology and compare them. The next step will be to analyze and statistically process this proteome data, focusing on differential expression between the restricted and control animals.

CONCLUSION: With these partial results, it was possible to cover some interesting aspects of RENCA proteome and discuss the biologically relevant functions associated with them. Further, we hope to find out if there is a connection between sleep restriction and tumor development that can be described at a molecular level.

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