Theme: Redox and Metals in Biology
Abstract 1487

Fpr2-/- mice fed alcohol had reduced hepatic expression of coagulation factors and antioxidants, as identified by proteomic analysis

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Keywords: alcohol-associated liver disease, Fpr2, zinc, glutathione

Alcohol-associated liver disease (ALD) is a global healthcare problem with limited treatment options. Recently, Fpr2-/- (formyl peptide receptor 2 knockout) mice were shown to develop exacerbated alcohol-induced liver injury and inflammation, but the associated mechanisms remain elusive. Objective: To identify hepatic proteomic changes associated with exacerbated EtOH-induced liver injury in Fpr2-/- mice. Methods: A proteomic analysis was conducted on liver homogenates from WT and Fpr2-/- mice (n=3/group) that were fed either a control diet (pair-fed mice, PF) or a 5% ethanol (EtOH)-containing diet for 4 weeks followed by a single EtOH binge (5 g/kg). Liver antioxidants including Zinc (Zn) and total glutathione (GSH) were measured via colorimetric assay. Hepatic gene expression was assessed by RT qPCR analysis. Data were compared using a one-way ANOVA with a Tukey multiple comparison test. Results: There were 977 downregulated and 45 upregulated significant protein changes between Fpr2-/-EtOH and WT EtOH mice. Among the downregulated proteins in Fpr2-/-EtOH vs WT EtOH mice were the blood coagulation factors, F2 (mRNA & protein) and F9 (protein). Zn binding proteins, MT1 (protein) and MT2 (mRNA & protein), were significantly upregulated in Fpr2-/- EtOH vs WT EtOH mice. Increased expression of these Zn-binding proteins was associated with decreased free hepatic Zn in Fpr2-/- EtOH vs Fpr2-/- PF mice. Lastly, the rate limiting enzyme for glutathione synthesis, GCLC, and total liver GSH were downregulated in Fpr2-/-EtOH vs WT EtOH mice. Conclusions: These data suggest that loss of Fpr2 in the context of ALD may lead to impaired hepatic Zn and glutathione antioxidant system, contributing to oxidative stress and hepatocellular death. In addition, Fpr2-mediated regulation of F2 and F9 protein expression appears to be compromised which may contribute to ALD- induced coagulopathy. Further investigation is required to understand precisely how FPR2 impacts these processes in ALD.

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Theme: MAC: Cancer Biology
Abstract 1531

Mass spectrometry-based proteomic analysis of glioblastoma-derived extracellular vesicles and healthy brain cells for biomarkers identification

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Keywords: Glioblastoma, Exosomes, Neurobiology, Diagnostics, Early detection

Cellular communication within the brain plays a crucial role in maintaining physiological equilibrium as well as triggering the onset of diseases. Extracellular vesicles (EVs) are released by donor cells and encapsulate RNA, DNA, and proteins that influence gene and protein expression upon uptake by recipient cells. Transcriptomic and proteomic cell alterations have been associated with the development and progression of glioblastomas. This study aims to identify EV-derived glioblastoma biomarkers through a comparative mass spectrometry-based bottom-up proteomic analysis of glioblastoma LN-229 cell line and healthy human neurons, astrocytes, and endothelial brain cells (HEBC), therefore, advancing the understanding of the underlying mechanisms of disease progression and paving the way for the development of liquid-biopsy-based noninvasive diagnostic approaches. EVs were extracted from the conditioned media of LN-229, human neurons, astrocytes, and endothelial brain cell lines via polymer precipitation (Thermo Scientific, cat. # 4478359). Trifluoroacetic acid followed by neutralization with Tris buffer was used for rapid proteomic extraction. The protein samples were digested with trypsin and the peptides were purified using Pierce™ C18 Tips (Thermo Scientific, Cat. # 87782). Fifty nanograms of peptides were injected for mass spectrometry analysis on the timsTOF Pro 2 instrument. Each experimental group included three biological replicates. Data analysis was performed using Spectronaut software (version 18.3, Biognosys) for processing mass spectrometry data acquired using data-independent acquisition methods. The statistically significant proteins were annotated using the STRING database. Gene Ontology enrichment analysis was conducted to analyze the biological significance of the overexpressed proteins. Comparative analysis of astrocytes and LN-229 EVs showed that the glioblastoma fraction displayed an average of 1,499 proteins. The proteomic profile of human astrocyte-derived EVs featured 1,301 proteins. A total of 48 proteins showed statistically significant upregulation in glioblastoma EVs compared to human astrocytes. A subset of 13 proteins are associated with glioblastoma tumorigenesis and metastasis. Analysis of EVs derived from both glioblastoma and healthy human astrocytes, neurons, and HEBC was performed to identify biologically significant biomarkers. EVs released by the human brain include a mixture of EVs originating from

neurons, astrocytes, and HEBC. Therefore, accurate biomarker identification requires a comparison of the proteomic expression profiles between glioblastoma and a healthy, diverse background. The analysis focused on identifying glioblastoma EV-derived proteins that were consistently overexpressed across at least two of the neurons, astrocytes, and HEBC experimental groups. A subset of 20 proteins was identified as potential biomarkers of glioblastoma. The STRING database annotation indicated that these proteins play a significant role in promoting cancer development and metastasis including increased protease activity, angiogenesis, and regulation of immune and redox response. This study sheds light on the role of EVs in glioblastoma progression and metastasis, and their biomarker's potential for early detection and targeted therapeutic interventions in glioblastoma patients.

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Abstract 1590

Development of a method to tune the stoichiometry of central dogma-related proteins synthesized in vitro

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Keywords: self-replicating artificial cell, in vitro reconstitution of the autonomous central dogma, parallel reaction monitoring, Escherichia coli

Background: The creation of a self-replicating artificial cell is an essential goal to understand the self-replication of life. To achieve this goal, it is necessary to reconstitute the autonomous central dogma, a system in which DNA replication, transcription, translation, and ribosome biogenesis are driven autonomously from DNA, outside a living cell. However, it is difficult to reconstitute the autonomous central dogma in vitro because a number of central dogma-related factors (26 DNA replication factors, 5 transcription factors, more than 100 translation factors) need to be synthesized in a proper stoichiometry and these newly synthesized factors must autonomously drive the central dogma system. In this study, we have tried to develop a method to easily tune the stoichiometry of central dogma-related proteins synthesized in vitro. Methods: To tune the stoichiometry of multiple central dogma-related proteins, it is necessary to quantify the production profile of these proteins. Therefore, we used Parallel Reaction Monitoring (PRM), which can quantify multiple target proteins simultaneously with high stability and sensitivity. To quantify the in vitro synthesized proteins, we isotopically labeled nascent proteins with 13C- and 15N-labeled Lys and Arg. Next, to reconstitute the autonomous central dogma in vitro, we simultaneously produced several autonomous central dogma-related proteins in vitro and quantified their production profile by PRM. Finally, based on the results, we tried to optimize the production profile by changing the input ratio of each gene. Results and Discussion: As an application of the developed method, we attempted to quantify and tune the stoichiometry of 26 DNA replication-related proteins (DRPs) synthesized in vitro. First, we simultaneously expressed 26 genes encoding DRPs by adding the genes at the ideal ratio of DRPs [1]. Then, using the developed PRM, we calculated the ratio of the actual production level to the ideal production level for each DRP and called it the satisfaction value. As a result, the coefficient of variation (CV) of the satisfaction values was 0.90, indicating an unbalanced stoichiometry of the in vitro synthesized DRPs. We then adjusted the input ratio of each gene to be equal to the reciprocals of the satisfaction values. As a result, the CV of the satisfaction values was reduced to 0.56, indicating that the stoichiometry of each protein was greatly improved. In conclusion, we have developed a proteomics