H136 Brain Damage and MicroRNA (miRNA) Dysregulation: An Experimental Study

Francesco Sessa, MS, University of Foggia, Forensic Pathology, Foggia 71100, ITALY; Francesca Maglietta, MD*, University of Foggia, Forensic Pathology, Foggia 71122, ITALY; Giuseppe Bertozzi, MD*, Department of Forensic Pathology, Foggia 71121, ITALY; Luigi Cipolloni, MD, PhD, Department of Forensic Medicine Sapienza Rome, Rome 00161, ITALY; Monica Salerno, MD, PhD, University of Catania, Forensic Medicine, Catania 95123, ITALY; Pietrantonio Ricci, PhD, University of Catanzaro, Catanzaro, ITALY; Cristoforo Pomara, MD, PhD, Catania, Foggia, ITALY*

Learning Overview: After attending this presentation, attendees will have a better comprehension of miRNAs as a useful tool, both in the clinical setting and in medicolegal investigation. Particularly, this experimental study focused on brain damage, analyzing the miRNAs expression values in four selected groups of cadavers.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing evidence about the expression levels variation of the specific miRNAs (miR-21, miR-34, miR-124, miR-132, and miR-200b) involved in the control of important target genes that regulate the neuronal apoptosis and neuronal stress-induced adaptation.

Brain damage and/or dysfunction as a sequelae of multiple different conditions is considered an important field of research for the scientific community. Recent studies have focused on side effects related to aging of the Central Nervous System (CNS). Moreover, a growing number of investigations have been conducted, analyzing the CNS effects of stroke and drug use/abuse. The identification of specific circulating and/or tissue biomarkers that could indicate brain injury remains challenging, however. Since 2007, miRNA technology has become an integral part of research; the scientific community has frequently investigated utilizing miRNA as potential molecular biomarkers for several diseases. Today, miRNA dosage has become an essential tool in several clinical applications.

This experimental study focused on miRNA expression in cases of brain injury. MiRNA is a well-known diagnostic tool, both in the clinical setting and in medicolegal investigations. Previous studies have demonstrated that specific miRNAs (i.e. miR-21, miR-34, miR-124, miR-132, and miR-200b) control important target genes involved in neuronal apoptosis and neuronal stress-induced adaptation. Thus, in this experimental setting, their expression was evaluated in four selected groups of decedents in which five males were selected for each group (i.e., Anabolic Androgenic Steroid [AAS]; proprionate testosterone) abuser, drug abusers (cocaine), ischemic-stroke-related deaths, and aging damage in elder decedents who died from other neurological causes).

Total RNA, including miRNAs, was isolated from Formaldehyde-Fixed Paraffin-Embedded (FFPE) samples using the RecoverAll™ Total Nucleic Acid Isolation Kit with minor modifications. For miRNA profiling, the TaqMan Advanced miRNA Assay was used. Complementary DNA (cDNA) was obtained following TaqMan® Advanced miRNA Assays User Guide (Publication number 100027897 Rev. C). Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was performed using the StepOnePlus™ Real-Time PCR System, and raw data were analyzed using the relative software (version 2.3). Expression fold changes were computed using the $2^{-\Delta\Delta Ct}$ calculation.

Study results demonstrated that drug abuser and AAS abuser groups showed a higher expression of miR-132 and miR-34, suggesting a specific pathway in consumption-induced neurodegeneration. Conversely, miR-200b and miR-21 dysregulation was linked to age-related cognitive impairment. Finally, ischemic stroke-related deaths were associated with an alteration in miR-200b, miR-21, and miR-124; significantly higher levels of this last expression were strongly sensitive for ischemic damage. Moreover, these results suggest that these expression patterns could be studied in other biological samples (e.g., plasma, urine) in subjects with brain injury linked to aging, AAS abuse, drug abuse, and stroke to identify reliable biomarkers that could be applied in clinical practice. Further studies with larger samples are needed to confirm these interesting findings.

Anabolic Androgenic Steroids (AASs), Brain Injury, MiRNA Dysregulation