B124 DNA Methylation Patterns as Markers in Forensic Investigation

Deborah Silva, MS, 118 Zamora Avenue, Apt 308, Miami, FL 33134; Joana Antunes, MS, Florida International University, 11200 SW 8th Street, Lab OE294A, Miami, FL 33714; Kuppareddi Balamurugan, PhD, University of Southern Mississippi, School of Criminal Justice, Hattiesburg, MS 39406; George T. Duncan, PhD, Broward County Crime Lab, 201 SE 6th Street, Rm 1799, Fort Lauderdale, FL 33301; Clarice Alho, PhD, PUCRS-FABIO, Av Ipiranga, Porto Alegre, BRAZIL; and Bruce R. McCord, PhD, Florida International University, Dept of Chemistry, University Park, Miami, FL 33199*

After attending this presentation, attendees will understand the application of epigenetics markers in forensic casework and the importance of this new tool of identification in the resolution of a crime scene.

This presentation will impact the forensic science community by providing crucial information on the development/validation of DNA methylation markers for forensic use. The new and improved technique for the identification of cell type and age will provide the community with new and improved methods to interpret the crime scene.

Body fluids recovered from crime scenes are considered one of the most important types of evidence in forensic cases. DNA obtained from body fluids can be used to identify the donor of the biological material but as presently used it cannot reveal the tissue source or the possible age of the donor. Determining the type and origin of the fluid can provide important assistance in reconstructing crime scenes. DNA presents the ideal source for identification of tissue type since it provides quantitative results and is more stable than RNA. In addition, the extracted DNA target is already present in the laboratory.

DNA methylation is an epigenetic modification involved in transcriptional regulation.[1] It is known that methylation is important in cell differentiation and genomic loci are differentially methylated between tissues. Because of this, different methylation patterns between tissues and cells can provide the basis of an assay for body fluid identification. The ability to determine the age of the sample donor based on DNA would also be a powerful tool for forensic investigation. Human ageing is associated with epigenetic modifications such as DNA methylation. Several studies have investigated biomarkers for ageing which can be used to track donor age, presenting practical implications in forensic analysis.[2,3]

The most common body fluids found at crime scenes are blood, semen, and saliva. A set of epigenetic markers, C20orf117, ZC3H12D, BCAS4, and FGF7, has been developed which produce unique and specific patterns of DNA methylation that can be used to identify these body fluid types; however, to ensure the efficiency of these epigenetic markers, developmental validation studies needed to be performed to determine the conditions and limitations of this new tool for forensic analysis.[4] For this research, all validation studies were performed according to Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines. Also, five genes previously found to be DNA methylation age-associated, NPTX2, TRIM58, GRIA2, KCNQ1DN, and BIRC4BP, were tested for prediction of age using a variety of body fluids and a pyrosequencing system.[3] DNA was extracted and bisulfite conversion was performed using the Epitect® Bisulfite Kit. Bisulfite modified DNA was then amplified using specific primers for methylated target regions. The last step was analysis by pyrosequencing. All pyrosequencing reactions were performed using the PyroMark® Q24 Pyrosequencer. Data analysis was performed using the PyroMark® Q24 assay software for CpG methylation quantitation and the corresponding percent methylation values for each site and the data were displayed as a pyrogram.

The versatility of these new markers will be presented by showing the results of validation studies on sensitivity, human specificity, age, and mixture resolution. When testing the markers using different species samples, amplification occurs not only for human DNA but for some animal samples; however, when the amplified DNA is pyrosequenced, the sequencing primer increases the specificity and eliminates results from any non-human samples. All the other samples show a pyrogram equivalent to the one obtained for the negative control. In another test, samples were degraded by being heated at different temperatures. All degraded samples showed good pyrosequencing results. In a study examining the effect of Polymerase Chain Reaction (PCR) inhibition, all samples were amplified and showed good pyrograms when hematin (0.08mM) and humic acid (0.24mg/mL) were added before bisulfite modification. When these same inhibitors were added after bisulfite modification and before PyroMark® PCR, there were no satisfactory results. This data indicates that the purification process at bisulfite conversion removes inhibitors during the washing steps. Sensitivity and mixture studies were also performed. In general, the results generated indicate a good perspective for the overall validation of the tested markers.

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