A44 Epigenetic Markers: A Forensic Tool for the Determination of Biofluids Present at Sexual Assaults and Other Crime Scenes

Tania Madi, BSc, 90 Southwest 3rd Street, Apartment 1414, Miami, FL 33130*

The goal of this presentation is to demonstrate to attendees a new method to identify the originating tissue source of DNA.

This presentation will impact the forensic science community by providing a novel tool that may be useful in the differentiation of biofluids and cell types such as saliva, blood, sperm, and epithelial cells.

Often in forensic cases, knowledge of the originating source of the DNA found at crime scenes is required. Although certain chemical and microscopic tests exist in tissue type identification, they are mainly presumptive with varying sensitivity and specificity. Therefore, the development of a method to identify the source of DNA found at crime scenes is imperative. This study explores the possible use of epigenetic markers in identifying DNA sources.

Epigenetic modification of mammalian DNA is a naturally occurring mechanism crucial for the function of the genome and its transcriptional regulation. Although the DNA sequence in each individual cell is identical, its epigenetic profile is not. Epigenetic modifications include methylation at CpG islands and histone deacetylation. These modifications play a role in regulating the transcription and expression of genes allowing cells to differentiate into functionally and metabolically specialized cell types. The study of epigenetics as a useful tool in forensic tissue identification has proven to be promising. In the current study, researchers present a set of epigenetic markers found to be differentially methylated in four common bio-fluids found at crime scenes: saliva, blood, sperm, and epithelial cells.

After PCR amplification, any methylation information is lost. It is therefore necessary to subject DNA to bisulfite conversion, a chemical treatment that converts all unmethylated cytosine bases in the DNA to uracil and subsequently to thymine bases during PCR. These base changes are then detected in downstream analysis such as PCR or sequencing. The detection of a thymine base at a CpG site indicates that the modification process was successful in converting the cytosine base and that the site was not methylated. In contrast, if a cytosine base persists during the modification process and a thymine base is not detected, the CpG site is likely methylated.

Samples of each tested biofluid were first taken from ten individuals. The DNA was extracted and the entire genome subjected to bisulfite conversion. Modified DNA was PCR amplified at several specific loci that are believed to be differentially expressed in these biofluids. Loci chosen contain a minimum of five CpG sites. The resulting PCR products were then sequenced using a Pyromark (Qiagen) pyrosequencer. Pyrogram peaks displaying the sequence and the percent methylation level at each CpG site were then analyzed and compared between the four cell types for methylation pattern differences. These profiles showed hyper or hypomethylation of one cell type relative to the other cell types. Using this method, epigenetic markers were identified to differentiate saliva, blood, sperm, and epithelial skin cells. The results of this study indicate the potential of a novel tool in identifying biofluids found at crime scenes.

Epigenetic, Pyrosequencing, DNA

 ** Presenting Author*