A87 Investigations on the Use of Tissue-Specific MicroRNA Markers to Determine the Wound-of-Origin of Bloodstains

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After attending this presentation, attendees will have an understanding of the limitations of bloodstain pattern interpretation in certain case circumstances and the potential use of tissue-specific microRNA assays to determine the relationship of discovered bloodstains to the homicide under investigation.

This presentation will impact the forensic science community by revealing a new approach to correlate bloodstains with injuries. The method offers a way to test suspects' alibis which cannot be accomplished with current methods. The presentation will additionally impact the forensic science community by demonstrating that molecular markers can provide information on the circumstances surrounding a crime.

The body of a homicide victim is oftentimes removed from the primary scene by the perpetrator and disposed of elsewhere. The location of the murder then becomes an important fact to establish in the investigation. Knowing where the murder took place can assist investigators in identifying suspects. Murders often result in significant bloodshed which can allow investigators to establish the location of the murder based on bloodstain pattern interpretation. However, the circumstances of other homicide cases are such that little blood is shed or even discovered due to the nature of the injuries or the act of cleaning by the perpetrator. Additionally, the suspected murder scene is often a place where the victim is known to have a history of physical activities—sometimes the suspected murder scene is the victim's residence. These circumstances can complicate investigations; because if small amounts victim's blood are found at the victim's residence or at a place where the victim visits, then the question becomes whether the bloodstains are related to the homicide or the result of some prior accidental injury. Unfortunately, current forensic methods used to correlate bloodstains with injuries are greatly limited when dealing with trace amounts of blood or bloodstains that have uninformative patterns.

However, in several cases the authors have been able to correlate bloodstains with particular wounds based on the cellular composition of the stains. In each of these cases, the victim's injury was a fatal gunshot wound to the head, and the body was found at a site away from where the homicide was thought to have occurred. However, several bloodstains from the victim were discovered at the defendant's house which was the suspected homicide scene. The defendant stated the blood was unrelated to the murder—the victim had previously cut his finger as a result of an accident. The size, shape, and distribution of the bloodstains were consistent with the defendant's alibi. However, each of the bloodstains contained visible pieces of tissues. These tissues were sectioned and stained for histological examination and were identified microscopically as brain. The presence of brain tissue in each of the bloodstains indicated the blood was shed as a result of head trauma. The finding refuted the defendant's alibi and was pivotal in the resolution of the case. Unfortunately, the finding of discernible pieces of tissue in bloodstains is rare. However, the authors hypothesized that trace quantities of wound-track cells are present in evidentiary bloodstains. However, the minute quantity of cells would render the histological approach impractical. The detection of these cells requires a method that is sensitive and specific. Sensitivity and specificity are properties of current forensic DNA typing methods; therefore, this research investigated a molecular approach to correlate bloodstains
with injuries.

The developed a PCR-based technique to detect trace amounts of wound cells in bloodstains is reported. In this proof-of-concept study, the laboratory rat was used as a model to investigate the use of tissue-specific micro-Ribonucleic Acid (miRNA) markers to distinguish bloodstains originating from different wounds. Specifically, the miRNA species, Rn_miR-124a_1, as a marker for rat brain tissue was studied. The basic procedure for the miRNA assay consisted of the following steps: (1) extraction of total miRNA from simulated head wound bloodstains using a rat blood brain mixtures using QIAGEN's miRNeasy mini kit; (2) synthesis of cDNA from miRNA with QIAGEN miScript Reverse Transciptase Mix; (3) amplification of the target miR124a-1 with Taq polymerase and oligonucleotide primers from QIAGEN miScript Universal Primer, and miScript Primer Assay; and, (4) identification of the miR124a-1 cDNA using the QuantiTect SYBR Green PCR Master mix fluorescence detection with the Rotor-Gene Q Real-Time PCR Detection System.

Preliminary studies included the optimization of the detection assay and the evaluation of the specificity of the marker. Additionally, a procedure for the collection of bloodstains for use by this assay, and the stability of the marker under different environmental conditions was examined. Proof-of-principle was achieved by the ability to distinguish bloodstains produced by a gunshot wound to the head versus bloodstains produced by a gunshot wound to the chest with use of the assay. This research illustrates that molecular markers can reveal information about the circumstances surrounding the deposition of biological evidence. This research stems from limitations encountered with current forensic

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methods, and the use of this approach may enhance the successful resolution of forensic investigations and the administration of justice.

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