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Forensic MicroRNA (miRNA) Analysis of Skin Specific Markers

Natalie Coult, Mari L. Uchimoto, Graham A. Williams

Introduction

Conventional body fluid identification methods are typically presumptive tests. These tests have a large number of false positives and because of this they are not confirmatory. Messenger RNA (mRNA) profiling was developed as a body fluid identification method as it was determined that mRNAs are expressed in a tissue-specific manner. Unfortunately, given mRNA’s size (~200-300 nucleotides), using this technique may not be ideal for compromised or degraded samples often encountered in forensic casework. MicroRNAs (miRNA) are small, non-protein coding RNA molecules approximately 22 nucleotides in length. The first miRNA was discovered by Rosalind Lee et al. in 1993, whilst studying the lin-14 gene in *C. elegans* development. miRNAs are believed to control gene expression involved in metastasis, proliferation, apoptosis and differentiation. A large number of cellular pathways are affected by the regulatory function of miRNAs, the most notable of these pathways control oncogenic and developmental processes. It has also been found that miRNA processing defects can enhance tumorigenesis.

Many people argue that there is no need to test for the presence of a specific body fluid as the analysis of any DNA present can confirm a body fluid is present. This is true, DNA won’t be present if a biological material isn’t present, but the confirmation of the body fluid could prove crucial in the investigation of a case. There is a need to identify skin alongside other body fluids within forensic investigations, especially sexual offences. For example, it is not currently possible to distinguish between vaginal material and skin cells from, for example, fingernail scrappings. Thus, in order to develop a technique that is capable of doing so, it is necessary to be able to identify skin cells.

The aim of this project was to determine whether skin, saliva and vaginal material (VM) could be correctly differentiated from one another using microRNA analysis.

Method & Materials

**Sample Recovery**

**Sample Preparation**

**Total RNA Extraction** (QIAGEN RNeasy Mini Kit)

**Stem-Loop Reverse Transcription** (ABI TaqMan Small RNA Assays)

**Real-Time PCR** (ABI TaqMan Small RNA Assays)

**Data Analysis** (ΔCt calculation and analyses of variance)

Results

**Skin Cell Recovery Methods**

- Method A: a dry cotton swab
- Method B: a wet cotton swab
- Method C: using a blunt edge and a wet cotton swab

Method C was found to be the optimal method to use.

**Blood vs Saliva markers**

- Blood samples tested with hsa-mir-203 and VM with hsa-mir-126 show no significant expression levels of their specific miRNA probe/marker.

**Skin, Saliva, Blood & VM Samples**

- A significant difference exists between skin, saliva and VM samples.

**Conclusion**

- A significant difference between skin and saliva samples, and skin and VM samples, has been determined using hsa-mir-205 and 617, respectively.

Future Work

Future research will involve identification of a skin specific miRNA probe/marker which can differentiate between skin, saliva and vaginal material, without the need for a second or third miRNA probe/marker to be used. Future research will also incorporate the differentiation of seminal fluid, sample mixtures and a single stream method for microRNA analysis and DNA analysis.