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The species specific of 3 microRNA markers in saliva

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ABSTRACT

MicroRNAs (miRNAs) have been proven to exist in forensically relevant body fluids. However, we need to know whether the result would be influenced by other species' saliva. This study aims to analyze the expression levels of 3 miRNA markers in different species, including *Homo sapiens*,*Felis catus*, *Canis lupus familiaris*, *Cavia porcellus*, *Sus scrofa*, *Mus musculus*, *Oryctolagus cuniculus*, *Bos taurus*, *Capra hircus*. MiRNAs were extracted using Qiagen kits from 9 kinds of different species' saliva. Reverse transcription and quantitative PCR was performed with SYBR® Green with water as a negative control and U6b as a reference. Some of target miRNAs were found to be expressed in different body fluids of both human and animals. Saliva microRNA markermicroRNA200c (miR-200c) which is highly expressed in human being saliva [1] has a high level of expression in other species. Our results suggest that species specificity should be analyzed before identifying crime scene evidence because the stains may be mixtures with some other species. We propose to carry on species specific research when seeking a new candidate miRNA marker for forensically relevant human fluids.

1. Introduction

MicroRNAs, functioning as regulators in a cell, are a class of noncoding RNAs and reportedly more stable than messenger RNA (mRNA) due to its small size [2,3]. MicroRNA has been introduced into the forensic field by Hanson et al. [4] since 2009. Recently, many researchers have reported that microRNA exist in forensically relevant body fluids, such as blood, saliva, semen, menstrual blood and vaginal secretion [1,4–8]. This research usually focused on human body fluids, and it is therefore necessary to perform species specificity experiments to identify any issue specific to the presence of animal body fluids. This paper aims to analyze the expression level of three saliva microRNA markers in different species, including *Homo sapiens* (human beings), *Felis catus* (cat), *Canis lupus familiaris* (dog), *Cavia porcellus* (guinea pig), *Sus scrofa* (pig), *Mus musculus* (mouse), *Oryctolagus cuniculus* (rabbit), *Bos taurus* (cow) and *Capra hircus* (sheep).

2. Material and methods

2.1. Sample and markers

Human saliva samples were acquired from healthy volunteers and animal saliva samples were collected from healthy animals using sterile swabs. Samples were then dried in a cool room temperature environment before storage. We chose 3 saliva microRNA markers—microRNA200c (miR200c), microRNA205 (miR205) and microRNA658 (miR658) which were reported earlier [1–5] and took U6b as the reference gene [9]. The sequence of those miRNAs was listed as follows:

MiR200c: UAAUACUGCCGGGUAAUGAUGGA;
MiR205: UCCUUCAUUCCACCGGAGUCUG;
MiR658: GGCGGAGGGAAGUAGGUCCGUUGGU;
Normalization-RUN6b: CTGCGCAAGGATGACACGCAAATTCGTGAAGCGTTCCATATTTTT.

2.2. Kits and methods

MicroRNAs were extracted from saliva samples with the miRNeasy Mini Kit (Qiagen, Germany) following the manufacturer's protocol and then quantified on the Nanodrop 1000 spectrophotometer (Thermo). The cDNA was synthesized by miScipt II Kit (Qiagen, Germany) according to the manufacturer's protocol.

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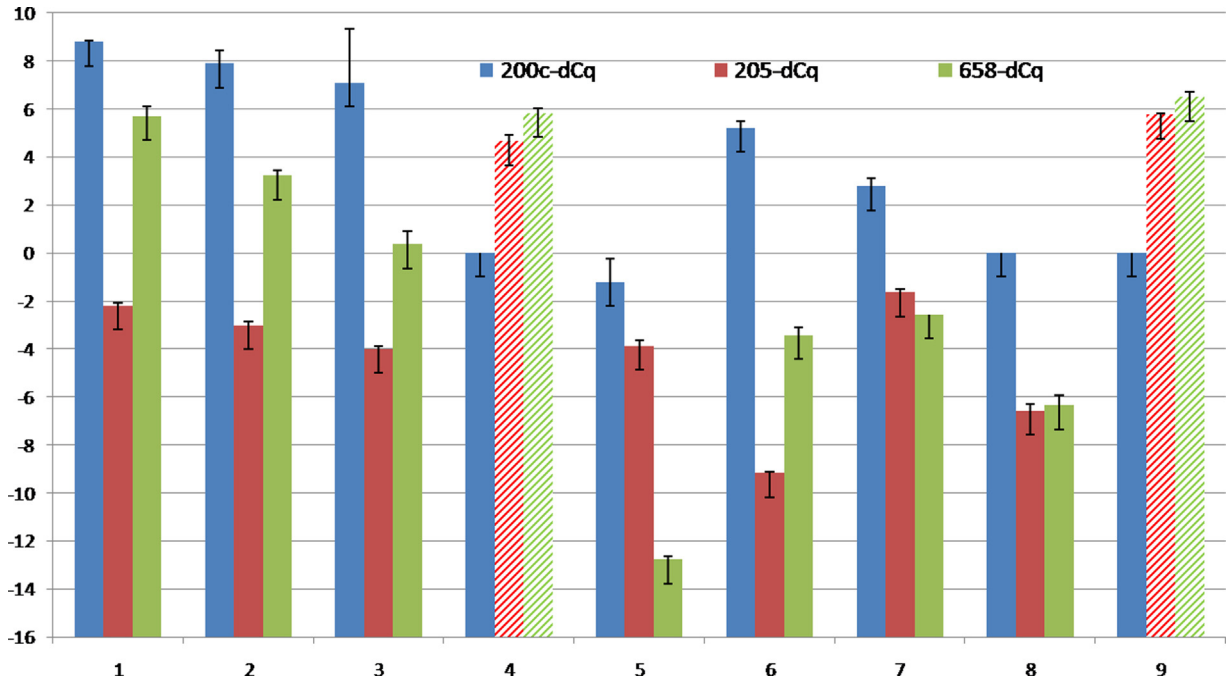

This study used ABI 7500 Real-Time PCR (Life Technologies) to measure the expression level of saliva microRNA markers with the MiScript SYBR® Green PCR Kit (Qiagen, Germany) following the manufacturer's instruction. Each sample was reanalysed 2 more times for reproducibility and negative and positive controls were included.

3. Results and discussion

The $dCq = Cq_{target} - Cq_{u6}$, and it was applied to all samples. If the reference gene U6b was not detected in some species, $dCq = Cq_{max} - Cq_{target}$, ($Cq_{max}$ was 40). The results in Fig. 1 show the expression of miR200c, miR205 and miR658 in saliva samples. The comparison of dCq values represents the different expression level of 3 saliva microRNA markers.

From Fig. 1, we can see that miR200c, miR205 and miR658, recognized as human saliva specific microRNA markers [1–5], express well or even better in animal saliva than human saliva, thus suggesting that the miR200c, miR205 and miR658 markers are not specific for human saliva. Moreover, the dCq value of miR200 c in sample 1 was higher than for other samples, which means animal saliva (samples 4–9) showed higher expression levels than human saliva (sample 1). Besides, we can conclude that some animal saliva (samples 2 and 3) had a similar expression level with human saliva (sample 1). Consequently, the saliva microRNA profiling results may appear as false positive result with the materials found in crime scene especially when there is no species identification.

From the data we collected, we can conclude that the saliva microRNA markers were not specific for human beings. It indicates animal stains would impact microRNA profiling results. Therefore, species specificity should be tested before identifying stains found at the crime scene to avoid false positive results. And this species experiment should be expanded to study other forensically relevant body fluids and increase sample number and diversity.

4. Conclusions

Our results suggest that species specificity experiments need to be conducted before identifying stains found at a crime scene are analysed with microRNA tests to avoid false positive results because the stains may be mixed with some other species' body fluids or human body fluids may be absent. We strongly propose to carry on species specific research when seeking a new candidate miRNA marker for forensically relevant human fluids.

Conflict of interest

None.

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