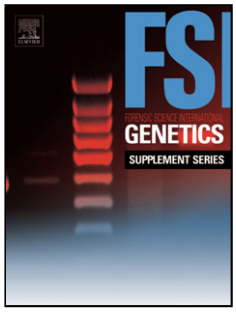

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Expression changes of microRNAs in menstrual blood samples of different menstrual cycle collection days

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Abstract. Three miRNAs (miR-141-3p, miR-497-5p, miR-143-5p) have been screened out and confirmed to be promising markers to distinguish between menstrual blood and peripheral blood by our study. However, studies reported the expression levels of miRNAs might be different in menstrual blood samples of different menstrual cycle collection days. To evaluate the dynamic changes of expression levels of these three miRNAs in menstrual blood samples from different menstrual cycle collection days, 51 menstrual blood (day 1-5 of menstrual cycle) samples were collected and detected. Total RNA was extracted then corresponding cDNA was prepared and three TaqMan Assays were run in triplicate using the TaqMan universal PCR Master Mix II without UNG (Life Technologies, USA). Bioinformatics analysis was used to search target genes of three miRNAs attempting to explain this observations. After comparing with the expression levels of previous peripheral blood samples, we found that mir-141-3p and miR-497-5p were still reliable miRNAs to distinguish menstrual blood from peripheral blood after the sample size was enlarged.

Keywords: Forensic science, menstrual blood, body fluid identification, microRNA

1. Introduction

Identifying menstrual blood is crucial for crime reconstruction [1] and may determine the nature and severity of the case, especially in sexual assaults. Blood is a body fluid that always means traumatic injury, while menstrual blood appears more often in sexual assaults [2]. Recent developments in nucleic acid detection methods have expanded the molecules available for forensic body fluid identification. The intrinsically small size and tissue-specific expression pattern make miRNAs less prone than mRNA to aggressive environmental factors that are regularly encountered in forensic scenes, which implies that miRNAs may be an ideal biomarker for body fluid identification.

Several studies have selected body fluid-specific miRNA markers via array screening and four miRNA markers targeting menstrual blood were confirmed. However, the reproducibility of the confirmed miRNAs was poor. Among the forensic body fluids, blood has been studied the most intensively; several mature miRNAs have been proposed to distinguish blood (including menstrual blood) from other body fluids. Menstrual blood contained a mixture of three distinct body fluids: blood, vaginal secretion and fluid of the late secretory phase of the uterine endometrial lining [2]. Moreover, whole blood accounts for only 30-50% of the total flow in most women [3]. There should have miRNAs that distinguish these two haemal body fluids.

Earlier, we searched miRNAs that distinguish menstrual blood from peripheral blood and 3 miRNAs were confirmed in TaqMan hydrolysis probes [4]. Twenty five samples were used in that study and most were from 2 or 3 day of the menstrual cycle. Previous research showed expression of miRNAs varied significantly throughout the menstrual cycle and the fluctuation may influence the application of miRNAs in body fluid identification [5]. Here, to confirm the reliability of these markers throughout the menstrual cycle, 51 menstrual blood samples (day 1-5 of the menstrual cycle) were collected, detected and compared with that of previous 10 peripheral blood samples.

2. Material and methods

2.1 Sample collection and RNA preparation

Menstrual blood samples were obtained with the approval of the Ethics Committee of Sichuan University (West China University of Medical Sciences). Menstrual blood samples were collected from 13 unrelated volunteers of the Chinese Han population living in Sichuan Province who gave written informed consent. Menstrual blood (day 1-5 of the menstrual cycle) was collected from the vagina using sterile gauze and dried at room temperature. The age range of donors of all the menstrual blood samples was 18-42. All samples were stored at -80℃ until extraction.

Total RNA was extracted from 2.5 cm^2 gauze containing menstrual blood using the miRNeasy Mini Kit (Qiagen, Germany). RNA quality and quantity was measured with a NanoDrop ND-1000 spectrophotometer.