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Mini Review

Molecular Marker for Body Fluids: Forensic miRNA

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Abstract

When found, collected, and correctly recognised, body fluids can be a valuable tool in forensic investigation. Numerous techniques, including those based on serology, were employed over a long period of time, but it was difficult to ignore their lack of sensitivity and specificity. MiRNA profiling exploded with a great potential to be utilised to identify evidences such urine, blood, menstrual blood, saliva, semen, and vaginal secretions in order to divert attention from the issue. MiRNAs are 20–25 nt short RNA structures that have characteristics that make them less susceptible to breakdown processes than mRNA. This is crucial since biological evidence may be exposed to various harmful environmental elements at a crime scene. A few particular miRNAs have recently been identified by published research, although their findings were not always repeatable by others, which may be the result of varied workflow techniques used for their profiling studies. Given the present surge in interest in miRNAs, it is crucial to recognise any potential limitations of miRNA profiling, however there aren't any research that clearly demonstrate this. . This review makes the claim that it has compiled all available data and evaluated a wide range of variables that could potentially cast doubt on miRNA profiling, including methodological approaches, environmental factors, physiological conditions, gender, diseases, and sample preservation. Although significant progress could yet be achieved, we pretend to emphasise one conceivable response to the fundamental query: Is miRNA profiling a forensic biomarker for identifying bodily fluids?

Keywords: Forensic science • Forensic serology • Body fluids • miRNA profiling • Biological biomarkers

Introduction

Small non-coding RNAs known as miRNAs, which have a length of about 22 nucleotides, appear to regulate a large portion of human genes when paired with the RNA-induced silencing complex. When this occurs, miRNAs influence gene regulation by inhibiting protein synthesis or degrading the mRNA by cleavage. They are involved in a number of cellular processes, including apoptosis, development, differentiation, and proliferation, and are well conserved in eukaryotic organisms. Numerous research conducted in recent years have demonstrated the importance of miRNA profiling as a method for identifying bodily fluids in forensic sciences and as a tool for understanding gene regulation mechanisms such as development mechanisms and gene regulatory networks. In 1993, Ambros and colleagues discovered the first miRNA, miR-lin-4. They noted that this miRNA controlled the timing of developmental processes in the C. elegans larval stages, and they found that Lin-4 does not actually code for proteins but rather for short RNAs. Later, Pasquinelli et al. described a second miRNA, let-7, and various species validated its occurrence in human tissue. This finding showed that these miRNAs have been maintained across lineages, providing the first indication of a more widespread problem [1]. DNA profiling can identify people based on their unique DNA signatures, but it cannot specify the kind and origin of the evidence. Based on the idea that every type of bodily tissue has a unique RNA signature, mRNA profiling has gained popularity as a useful method to pinpoint pertinent human body fluids. The mRNA's susceptibility to degradation by physical or chemical causes has always been a challenge, notwithstanding the success of mRNA profiling. Hanson et al. introduced miRNA profiling to the forensic sector in 2009, finding that miRNAs can recognise various body fluids through miRNA fingerprints. For instance, characteristic profiles of miRNAs may enable the differentiation of human body fluids such as blood, menstrual blood, semen, saliva, and vaginal secretions from other human tissues when a particular miRNA is specific to a human tissue and is absent from other samples or when its concentration is noticeably elevated and low in other samples. The RISC complex's catalytic argonaute proteins are in charge of the biological process known as RNA interfering. Their close association with miRNA renders them far more resistant to degradation than mRNA, which results in a higher capacity for discrimination, particularly under difficult circumstances. Through this research, we made the effort to compile all material that has been made so far about the use of miRNA as a potential biomarker for identifying body fluids. Recognize any factors, nevertheless, that might limit its utility as a biomarker [2].

miRNA as potential body fluid biomarkers

Body fluids are an important source of information that forensic pathologists and researchers can use to help solve crimes, identify criminals, and explain how someone died or was injured. Biological traces need to be identified after being found and recovered, although some biological stains are undoubtedly difficult to distinguish, such as venous blood from menstrual blood. There are numerous other procedures that have been employed over the years to identify body fluids, including chemical testing, immunological tests, microscopy, and spectroscopic techniques. However, some of them, like luminol for blood, are presumptive. Molecular genetics-based research is now being done to examine miRNA's potential as a biomarker, but can miRNA really be used as a reliable diagnostic for bodily fluids? Biomarkers should, in theory, be able to satisfy a large number of essential requirements. It must be possible to analyse using non-invasive techniques, have a long half-life in samples, be impervious to physical or chemical influences, be specific to a tissue, but most importantly, it must be a quick, easy, accurate, repeatable, and affordable procedure. If Hanson and colleagues were the first team to apply miRNA to the identification of body fluids in the forensic sector, other authors rapidly turned their attention to miRNA profiling. shows a list of all miRNAs that have been proposed by various authors as potential biomarkers for body fluids. The fact that practically all of the research carried out had disparate findings is shown in this figure. Only a small number of miRNAs are supported by two or more reports [3].

MiR-16 may be a potential biomarker for blood stains because studies conducted by Hanson, Zubakov, Wang, and their respective coworkers tend to indicate that venous blood is the only type of blood that miR-16 is specific to. MiR-205 was established as a reliable salivary biomarker by three separate research, while Wang et al. came to the opposite conclusion, contending that miR-205 may be epithelium-specific rather than saliva-specific. Urination is a typical vandalism act that can be a source of trace DNA and is also very useful for drug testing, especially for illicit drugs. Important processes for miRNA profiling include sample processing, RNA extraction, and sample selection. Degraded RNA can make it more difficult to identify

and quantify particular miRNA, which increases the likelihood of getting accurate results. However, it is now possible to obtain high-quality miRNA from fresh tissues, cell lines, plasma, and serum in addition to other bodily fluids, ensuring the sample's quality and quantity—both of which are important factors that have a big impact on the outcome. An essential step in the overall profiling procedure is sample collection. A sample may become contaminated during collection with elements other than the fictitious proof, such as ground soil, germs, etc. Can previously extracted miRNA continue to be in good form after being stored for a lengthy period of time, keeping in mind that criminal investigations can last for several years? Only a few research used forensically relevant body fluids to emphasise this subject, however some work was done using human serum. The study evaluated the impact of storage in human serum at 80 8C (with and without thaw), 20 8C (with and without thaw), and room temperature using four miRNAs.

It's interesting to note that mir-451, one of the four miRNAs taken into consideration, is also regarded in venous blood. The authors came to the conclusion that there was no discernible variation in miRNA levels between 80 8C and 20 8C during short-term storage (10 days). However, miRNA levels were significantly reduced but still detectable when kept at ambient temperature. Additionally, repeated freeze and thaw cycles considerably lower miRNA levels than continuous storing, even if this conclusion appears to be at odds with the findings of other authors' study. No significant variations in miRNA levels were found between 80 8C and 20 8C for mid-term storage (20 months), but certain individual miRNA were adversely impacted by those circumstances. Finally, they investigated the impact of long-term storage at 20 8C and noticed a minor drop between 2-4 years; but, after 6 years of storage, a considerable decrease in miRNA levels was noticed, which only gets worse over time. In conclusion, miRNA in human serum appears to be practically unaffected by freezing temperatures (20 8C) for at least 2-4 years. However, similar research is needed for other human body fluids as they might not respond the same manner to such low temperature preservation [4].

miRNA profiling–methodologies

With more or fewer than 22 nucleotides in length (the size of a typical primer), the traditional primer binding is impossible. MiRNAs' unique properties make miRNA profiling a difficult game. A smaller primer must be developed in order to circumvent the issue, which necessitates a lower melting temperature and eventually reduces the PCR's effectiveness. Additionally, the short length and variation in GC contents provide a broad range of melting temperatures, which presents another difficulty for its accurate profiling. These days, miRNA profiling can be done using a wide range of techniques. MiRNAs can be detected utilising techniques such as Northern Blotting with locked nucleic acid, RNase protection experiments, and in situ hybridization. However, we choose to focus on the three most popular techniques now in use: RNA sequencing, qRT-PCR (quantitative reverse transcription PCR), and microarray (a hybridization-based method). It's interesting how varied combinations of techniques were employed by the groups whose work was taken into account for miRNA profiling for bodily fluid identification. Could the variety of outcomes be caused by those combinations? This theory was put forth by Zubakov et al. after they attempted and failed to reproduce the findings of Hanson and colleagues for saliva, menstrual blood, and vaginal secretions. The basis for DNA microarray technology is a single strand of DNA (the probe) having the capacity to attach to a complementary strand of DNA (identified as target) [5].

Initially, cDNA is reversed into the targets' miRNA and fluorescently tagged. The microarrays are scanned to identify the fluorescence of the DNA probes, which may be detected using a fluorescent scanner, once the targets and probes (known sequences) successfully hybridized. The capacity to simultaneously investigate the expression of thousands of genes or their RNA products at a relatively low cost is one of the key benefits of microarray technology. The lack of specificity and sensitivity in comparison to other techniques like qRT-PCR or RNA sequencing is thought to be a real drawback that can skew the results, despite the method's high throughput. Another drawback is the size of the sample needed to do the experiment, which is a significant issue with forensic samples [6]. Furthermore, unlike qRT-PCR, microarray cannot be utilised for absolute quantification and only returns information on the genes that are included in the array itself, making it unable to identify novel miRNAs. As a high-throughput sequencing technique and one of the main methods for miRNA expression profiling, RNA sequencing (RNA-seq) is well-established. The reverse transcription of the miRNA to a cDNA library is the first step in the RNA-seq process. The cDNA library can be massively sequenced after it has been produced by applying adaptors to one or both of the sequences' boundaries. As a high-throughput platform for next-generation sequencing, RNA-seq is the only technique that can distinguish between miRNAs that are very similar, that is, separate miRNA that diverge by only one nucleotide, giving it a significant advantage over microarray and qRT-PCR technologies. Similar to microarrays, RNAseq cannot be used for absolute quantification, but it also has the fundamental drawback of requiring significant support from bioinformatics tools, which poses difficulties throughout the process [6,7].

Furthermore, despite declining over the past few years, the cost of RNA-seq per sample is remains considerable. Reverse transcription of miRNA to cDNA, followed by qPCR and subsequent real-time monitoring of the resultant products, are the foundations of quantitative reverse transcription PCR (qRT-PCR). Compared to the other two prior techniques, qRTPCR is far more sensitive and specific (particularly with TaqMan1-based detection) and can be utilised for absolute quantification. Because the amount of genetic material gathered in forensic investigations may be minimal, it's crucial to employ a method that uses just a small bit of RNA. Beyond being a well-established technique, qRT-PCR also has a low sample cost per sample and just modest RNA requirements. However, it also has a significant drawback in that it cannot find new miRNAs. For their experiment, Hanson, Zubakov, and the corresponding coworkers used qRT-PCR. Zubakov et al., however, were unable to duplicate several of the findings made public by Hanson and colleagues. SYBR1 Green and TaqMan1 were used, both of which were conducted on the same platform. SYBR1 Green recognises double-stranded DNA in an unspecific manner to detect PCR products, which might lead to erroneous positive results [8]. A TaqMan1 technique, on the other hand, depends on a certain hybridization between the probe and the target, which is necessary to produce a fluorescence signal. The qRT-PCR platform's sensitivity and specificity are increased with the addition of target and probe. m. Zubakov et al. speculate that the disparity in techniques may be one cause of the inconsistent outcomes. Additionally, when comparing the workflow of the authors taken into consideration for this review, it became clear that not only were the methodology used varied, but so was the normalisation gene they used.

There are no consensus normalizations for either of the approaches examined, despite the fact that they are both used to eliminate differences between samples and despite how vital it is to produce correct results. This emphasises how important it is to specify a clear workflow for miRNA profiling and standardise each technique [8].

Gender and miRNAs

We assemble data from published publications that concern miRNAs provided by authors as biomarkers for human bodily fluids in order to clarify whether or not they may be classified as such, and we display their various expression levels when altered by both biotic and abiotic stimuli. The gender-specific miRNA profiles will be the main topic of this section. Duttagupta et al research focused on miRNAs that have been found to be gender-specific in the general male and female population. A microarray examination of human plasma focused on a subset of four miRNAs (miR-548-3p, miR1323, miR-940, and miR-1292), which were found to be up-regulated in females, suggesting their potential to identify gender. It's interesting that none of these miRNAs saw a noticeable down-regulation. None of these miRNAs showed noticeably decreased expression . These outcomes would be predicted if the miRNAs in question, specifically mir-940, were sex tissue specific [9]. It makes sense that mir-940 is discovered to be up-regulated in female plasma compared to male samples because it is cervix-specific. The other three miRNAs, on the other hand, had no connection to the female sex tissues but were nevertheless up-regulated. Mir-130b and mir-18b had marginally greater quantities in male blood samples compared to female serum samples, according to research by Wang et al. from 2012. The scientists were unable to rule out the possibility that both miRNAs may have gender-association functions, it should be noted. Additionally, a 2013 study that examined the correlation between gender and the metabolic syndrome (MetS) and circulating serum miRNAs was also conducted. They found a substantial alteration in the miRNA profile, particularly for two miRNAs, let-7 g and miR-221, which were overexpressed in the serum of people with MetS and more prevalent in women ($P = 0.004$ and $P = 0.010$, respectively). Idealistic biomarkers should under no circumstances be

anticipated to change, but any exception to that rule may highlight its function as a biomarker. From a forensic perspective, it would be acceptable to examine whether those gender-related differences in miRNA levels may appear in other biological fluids since, if non-gender-specific miRNA tissue patterns alter, those miRNAs may not be appropriate as biomarkers [10].

Age-related miRNAs

Since miRNAs were discovered to be significant age-related regulators, investigations on them have grown. Lin-4 and let-7 from Caenorhabditis elegans were the first miRNAs to be genetically discovered. These and other miRNAs have been thoroughly investigated in this model and appear to demonstrate how lifespan regulation and longevity prediction are influenced. According to research by Boehm and Slack, overexpressing the miRNA lin-4 extends the lifetime of C. elegans, but lin-4 function loss has the reverse impact. Since many age-related miRNAs are conserved, Iba'n ez-Ventoso et al. hypothesised that their human homologs would be similarly controlled to affect ageing and aging-related pathologies. Idealistic biomarkers should never be changed in any way, by definition. Studying miRNA profiles is essential when both internal and external forces target them in significant forensic body fluids since this is why they are targeted. Only those that are sufficiently stable have the potential to be regarded as biomarkers. There are limited investigations on the age-related variations in miRNA levels in bodily fluids, despite the fact that many studies in mice, primates, and humans compare expression levels of miRNAs in younger and older tissues. MiR-34a and miR-93 were found to be more abundant in the middle-aged and old-age rat livers compared to the young rats when Li et al. investigated the expression of miRNAs in rat livers during ageing [11].

Discussion

Maes et al. also looked at the expression of miRNAs in the same tissue and found that miR-669c and miR-709 gradually rose from mid-age (18 to 33 months) to extremely old (33 months) mice, but miR-93 and miR-214 increased. By targeting transcription factors crucial for liver regeneration and detoxification, which were less active with ageing, the up-regulation of these miRNAs suppressed the expression of genes involved in oxidative defence. In another study, Li et al. investigated the role of miRNA regulation in the mouse brain during normal ageing. Their findings suggested that, similar to the liver, miR-22, miR-101a, miR-720, and miR-721 expression increased with age, starting in mid-adulthood and being seen as early as 18 months of age [12].

The majority of the miRNAs that were elevated were anticipated to target and express themselves in a way that was related to mitochondrial complexes [58, 59, 64]. Hamrick, Drummond, and their respective colleagues conducted more research on animal tissues such skeletal muscle. Few gene expression changes are exclusive to ageing, according to Somel et al. They investigated the expression of miRNAs in the prefrontal cortex of rhesus macaques and humans, and they found that 31% of the 373 miRNAs in humans showed substantial expression changes with ageing. Between humans and macaques, these results showed a strong positive connection (P = 0.020).

The first miRNA and noncoding RNA expression atlas of the growing human brain has just been created, with distinct expression patterns found in foetal, early postnatal, and adult brain tissue samples. Peripheral blood mononuclear cells from older American adults were found to have downregulated levels of a number of miRNAs (miR-103, miR-107, miR-128, miR-130a, miR-155, miR-24, miR-221, miR-496, miR-1538) compared to younger adults [13]. It is possible that transcriptional repression, deletion, mutation, epigenetic silencing, or faulty miRNA processing contribute to the loss of miRNA function throughout ageing, which raises the possibility that miRNAs and their expected targets could serve as diagnostic markers of ageing. 80 age-related miRNAs were found by ElSharawy et al. in blood samples, with 16 miRNAs up-regulated and 64 (80%) down-regulated in comparison to younger people. Their findings demonstrated that certain miRNAs, including miR-103, miR-107, miR-24, and miR-130a, previously found by Hooten et al. also emerged in this study. In addition, Hackl et al. discovered four miRNAs that are down-regulated and linked to ageing, among which miR-17, miR-20a, and miR-106a were verified by ElSharawy et al. It's noteworthy to note that some authors also see some of these miRNAs as bodily fluid indicators. MiR-130a appears to be colostrum-specific and an ageing biomarker; miR-20a and miR-106a, which were discovered in blood samples, were recognised as venous bloodspecific miRNAs but also age-related miRNAs. MiR-17, for instance, was recognised as a good biomarker for semen and a likely age-related miRNA [14]. Other study on bodily fluids has recently been conducted. In human serum, Hooten et alresearch .'s showed that miRNA expression changed differently as people aged. Because miR-151a-5p, miR-181a-5p, and miR1248 expression decreased significantly in this study, regardless of gender or race, it is possible that these molecules could be useful age-related indicators. All three miRNAs were anticipated to be key players in inflammatory pathways and crucial for organismal growth and survival, which raises the possibility that their expression may decline with age and lead to the emergence of diseases and phenotypes associated with old age. However, additional research would be required to confirm all of these findings so far. From a forensic perspective, these data purported to present an overall picture of miRNA profiling and the many variables that could influence it. Despite the undisputed potential of miRNA profiling, a number of questions have been highlighted by the research that has been done so far, and it would be smart to start looking into the answers. We would restate our results in response to the following two queries: Are miRNAs forensic indicators for identifying bodily fluids? Not today, at least. Can miRNAs serve as forensic indicators for identifying bodily fluids? Yes, we would say, but there is much work to be done before we can confidently respond to that question [15].

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Hannon G,. "RNA interference." nature 418.6894 (2002): 244-251.
2. Hutvagner G. and Simard M. "Argonaute proteins: key players in RNA silencing." Nature reviews Molecular cell biology 9.1 (2008): 22-32.
3. Santos J,et al. "Restoring TGFβ1 pathway-related microRNAs: possible impact in metastatic prostate cancer development." Tumor Biology 35.7 (2014): 6245-6253.
4. Várallyay E, and Havelda Z. "Unrelated viral suppressors of RNA silencing mediate the control of ARGONAUTE1 level." Molecular plant pathology 14.6 (2013): 567-575.
5. Dias, Francisca, et al. "Renal cell carcinoma development and miRNAs: a possible link to the EGFR pathway." Pharmacogenomics 14.14 (2013): 1793-1803.
6. Jovanovic M, and Hengartner M. "miRNAs and apoptosis: RNAs to die for." Oncogene 25.46 (2006): 6176-6187.
7. Andrea P. et al., "Non-coding RNAs as regulators of embryogenesis." Nature Reviews Genetics 12.2 (2011): 136-149.
8. Tay Y. et al. "MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation." Nature 455.7216 (2008): 1124-1128.
9. Esteller, Manel. "Non-coding RNAs in human disease." Nature reviews genetics 12.12 (2011): 861-874.
10. David B. "MicroRNAs: genomics, biogenesis, mechanism, and function." cell 116.2 (2004): 281-297.
11. Ana T. et al. "Higher circulating expression levels of miR-221 associated with poor overall survival in renal cell carcinoma patients." Tumor Biology 35.5 (2014): 4057-4066.
12. Lee R. et al. "The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14." cell 75.5 (1993): 843-854.
13. Pasquinelli A. et al. "Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA." Nature 408.6808 (2000): 86-89.
14. Vennemann, Marielle, and Antje Koppelkamm. "mRNA profiling in forensic genetics I: Possibilities and limitations." Forensic science international 203.1-3 (2010): 71-75.

15. Hanson E. et al., "Identification of forensically relevant body fluids using a panel of differentially expressed microRNAs." Analytical biochemistry 387.2 (2009): 303-314.

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