CITATION
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Abstract

Interest in molecular based techniques increases in every field of forensic sciences. Use of microRNAs (miRNA) in forensic genetics is one of them. The aim of this article is giving a brief information about microRNAs and their use in forensic genetic applications. MicroRNAs are small noncoding RNAs and because of their important features such as tissue-specificity and high stability in specific conditions, they are used in various studies for forensic purposes. In the article, usage areas of miRNAs and promising results obtained from these studies are mentioned. Body fluid identification, organ tissue identification, determination of stain age, wound age determination, post mortem interval (PMI) identification, age estimation of an individual, lesion assessment in strangulation cases, identification of brain damage and determination the abuse of anabolic androgenic steroids are the subjects which potential use miRNA are examined. The limitations of miRNAs in the forensic genetics applications are also mentioned in the article.

Keywords: MicroRNAs, Forensic Sciences, Forensic Genetics

INTRODUCTION

In molecular biology, central dogma is a very important phenomenon and it indicates the flow of genetic information in cells. According to this phenomenon, information is transferred from DNA to RNA (mRNA) and this whole transfer is named as transcription. Secondly, the information is transferred from RNA to protein. Protein synthesis from mRNA is called translation. In protein synthesis, three nucleotide found in mRNA indicates one amino acid in produced protein. Therefore, even a single change in mRNA can affect the structure of produced protein [1]. There are many regulatory mechanisms that play role in central dogma and some of them can be seen in Figure 1 below.

Primary role of RNA is playing a role in protein synthesis. During this synthesis, there are three main types of RNAs which are messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). Additional to these RNA types, there are regulatory RNAs which one of them is microRNA.

MicroRNAs (miRNAs)

MicroRNAs are noncoding RNAs which their length is approximately 19-24 nucleotides. Lin-4 was the first discovered miRNA in 1993 by Lee et al [3]. Second miRNA (let-7) was discovered 7 years later [4]. After that, researches were accelerated and their regulatory mechanisms were started to understand. Today, approximately after 30 years from the first
discovery of them, there are undiscovered miRNAs. Primary mission of miRNAs is interfering to translation mechanism by binding the target sequences on messenger RNA. By doing that, production of proteins is prevented or changed [5].

**Figure 1.** Central dogma and regulatory mechanisms which play role in protein synthesis [2].

**Forensic Genetics Applications of MicroRNAs**

In forensic sciences, applications of molecular techniques are becoming widespread owing to developing technologies. Use of miRNAs is one of the new technologies which adapted for forensic sciences. Before going through the details of miRNA use in forensic sciences, brief explanation will be given about the importance of evidences recovered from crime scene and the answers of who/what/how questions, for forensic cases. As seen in Figure 2, each question which may be answered by evidences are linked together and obtained answers lead to solve of the crime. To illustrate, knowing the identity of a person by analyzing the evidence can lead to arrest of a criminal or identification of a victim. Additionally, in the case of novel technology use, information of individuals’ characteristics such as biogeographical ancestry, hair color, age may be obtained. By using that information suspect pool can be narrowed. Secondly, having an information about the nature of evidence is very important in cases. For example, identification of a stain from crime scene can change the way of case especially in sexual assault cases. Lastly, obtained evidences can give some information about the way that criminal activity takes place [6]. MicroRNAs can be adapted to solve of all three questions in distinct ways.

Although the first miRNA was discovered in 1993, application of them into forensic sciences took 16 years. First research study for forensic purposes was done by Hanson et al. about the identification of body fluids [7]. After that, studies in different topics in the concept of forensic sciences were started to conduct. In this article, body fluid identification, organ tissue identification, stain age determination, wound age determination, post mortem interval (PMI) identification, prediction of an individual’s age, lesion assessment in the case of strangulation, brain damage identification and determination of anabolic androgenic steroids abuse are investigated forensic applications.

**Figure 2.** Relationship of who/what/how questions with evidences recovered from crime scene for solving the crime [6].

Firstly, body fluid identification by analyzing miRNAs is the topic which most of the studies are being conducted in the concept of forensic sciences. Because of the tissue-specific characteristics of miRNAs, the first idea was identifying the body fluids [7]. In some cases, determination of body fluids in the crime scene is very important for completing the story of case. To illustrate, in sexual assault cases, determining the origin of blood (venous or menstrual) is a serious issue. If this determination can be accomplished by miRNAs as a routine procedure, it will be helpful for forensic cases. Furthermore, reliability of taken statements can be questioned with the help of this technique. When compared with routinely used techniques, miRNA profiling is less harmless and requires less sample sizes [8]. In an interesting study which conducted in University of New Heaven, DNA and RNA isolation were performed simultaneously from the same sample [9]. Study was conducted in small sample group (each of blood, semen, saliva and urine samples from five voluntaries). From all the samples, DNA and miRNA profiles were obtained carefully. They conclude that this method is promising. If this technique can be developed by using larger sample groups and adapted into routine, both the individual’s identity and the nature of body fluid can be identified at the same time. Age of stains which obtained from crime scene can provide significant information about order of actions or relationship of stain with the crime. At the same time, estimating the individuals age from biological evidences can provide valuable data. In one study, blood samples were investigated for age prediction and massive parallel sequencing technology was used for this purpose. Six age related miRNAs were tested and mean absolute error was found as 5.52 years for male, 7.46 years for female [10]. By increasing the number of tested miRNAs and sample group size, promising results can be obtained.
In another study, a team worked with both young and old donors and they found that most of the miRNAs (total 800 miRNA was investigated) are decreasing with age from 800 investigated miRNA profiles [11]. Organ tissue identification is another promising field in the application of miRNAs in forensic science. Especially in violence related crimes, identification of organ tissues can be quite valuable. In order to identify the crime tool, this application may be used because during violence related crimes, tissues of internal organs may scatter to crime tool or crime scene. In addition to verification of crime tool, relocation of dead body can be identified in the case of organ tissue findings [8]. Robustness of miRNAs to degradation may be helpful in extreme conditions in the event of relocation.

In addition to these topics, estimation of post mortem interval (PMI) is one of the research area of forensic miRNA studies. PMI represents the time interval passed from the death. Like in other areas, high stability and durability of miRNAs when compared with other biomarkers made research groups to think as ideal biomarkers for PMI estimation [8]. Determination of PMI especially important in bodies which are in unrecognizable states. In this case miRNA analysis in skeletonized remains may be helpful to determine PMI. In one study, expression level of two specific miRNAs in bone tissue with PMI was associated. With the increasing PMI, negative correlation was monitored with expression of miRNAs [12]. Another interesting study related with application of miRNAs in forensic science is lesion assessment in hanging cases. In this study, miRNA profiles of skin samples belong to bodies died because of hanging were analyzed [13]. When compared with control group, significant differences were observed with inflammatory response related miRNAs. By increasing the sample group size and investigated miRNA number, more reliable results may be obtained and time of hang marks (before or after death) may be determined. Like in many scientific fields, molecular markers are becoming more important. If more accurate results may be obtained, miRNAs can be used for determination of vitality in hang marks in the future [14].

Another study area related with application of miRNAs in forensic science is wound age determination. In one of the review articles, is has been indicated that wound vitality studies are low in number. In order to differentiate antemortem and postmortem wounds, researchers stated that role of miRNAs in inflammation should be understood in depth [15]. In terms of forensic pathology, age of the wounds is one of the most important questions which should be answered. Studies about use of biomarker in this area are premature. With the collection of adequate and reliable samples, more accurate and helpful studies may be achieved [16].

Because miRNAs are useful biomarkers thanks to their features, brain injury diagnosis is one of the research areas which miRNA studies are conducted. In one of the studies about brain injury, specific miRNAs were found related with cocaine consumption, age related cognitive impairment and ischemic damage [17]. The study group stated that various biological samples could be investigated in order to obtain more informative results. Additionally, larger sample size could be beneficial too. By using this technique, determination of brain injury may be achieved by molecular techniques. In another study, researchers mentioned that miRNAs are promising biomarkers for forensic applications but obtained results are still insufficient for necessary specificity for traumatic brain injuries [18].

Another interesting study related with application of miRNAs in forensic science is detection of anabolic androgenic steroid (AAS) abuse. In the case of AAS abuse, some adverse effects can be observed in different systems (e.g., decreased glucose in endocrine system, irregular menstrual cycle in female reproductive system, and so on) in our body. These adverse effects lead to organ damages. As a result of organ damages, dysregulations of miRNAs occur. In the forensic concept, these dysregulations were investigated. Some important features such as high stability in tough conditions and long detection time of miRNAs make them potential anti-doping testing method [19]. In one of the systematic reviews, it has been mentioned that use of miRNA for identifying AAS related sudden death cases are low in number. They stated that this application not only increase the reliability of diagnosis the death cause but could support the scientific field for revealing the AAS abuse related conditions [20].

**Academic Research Studies Conducted in Türkiye**

When academic research studies conducted in Türkiye are examined, according to YÖK National Thesis Center [21], miRNA studies are mostly conducted in medical field especially for treatment aimed usages. In addition to these, there are studies which examines drug and alcohol addiction relationship with miRNAs. When forensic science related studies are searched (miRNA studies were filtered according to research area as 'forensic'), there were only two theses. One of them is about body fluid identification and the other one is about investigation of miRNA expression profiles in their post mortem brain tissues of people who are MDMA addicts.

**Limitations of MicroRNA Applications in Forensic Sciences**

Like every forensic analysis, degraded and/or mixed samples are the most challenging issue in miRNA analysis. When compared with other biomarkers, miRNAs' short sizes can be advantageous in this case. In addition, second limitation is insufficient funds in this area. Because DNA profiling is one of the most used techniques in forensic sciences, RNA technologies are approached cautiously. Moreover, use of novel technologies such as new generation sequencing (NGS) for investigating the miRNAs is costly unlike conventional methods. As parallel to funds, expertise on RNA profiling is inadequate when compared with DNA profiling. Lastly, sufficient information about miRNA damage did not be gathered unlike DNA damage until now [8].
CONCLUSION
As seen in literature, miRNA studies are novel study area when compared with DNA studies. With the correct standardization and validation processes, number of trustable miRNA biomarkers can be increased in the future. Additionally, developing technology in our time should be evaluated in this research field. By the evaluation of novel technologies, unknown information can be obtained and/or known information can be understood deeply. RT-qPCR which is a RNA profiling method widely used right now, can be replaced with the novel methods as new generation sequencing (NGS) technologies when proper conditions are supplied. NGS is a costly technology but when compared with other sequencing technologies, it provides huge amount of data. In the case of miRNA studies which has many unexplored features, NGS technology can be very useful. Very efficient results which obtained from NGS can tolerate the cost of it. Because biosynthesis pathways of miRNAs are not unique, undiscovered features of them can be lightened by new technologies. With the addition of specialists in this field, analyses can be performed more accurate and faster.

Being tissue specific characteristic of miRNAs make them potential biomarkers in body fluid identification but except from this purpose they have potential in other forensic applications such as organ tissue identification, stain age determination, wound age determination, post mortem interval (PMI) identification, prediction of an individual's age, lesion assessment in the case of strangulation, brain damage identification and determination of anabolic androgenic steroids abuse.

With the increasing number of studies in this research fields can provide very useful information in forensic sciences. Additionally, as mentioned in body fluid identification, simultaneous extraction of both DNA and RNA may be very useful technique in forensic sciences. Because forensic evidences obtained from crime scenes are usually very limited in size or degraded, useful information can be acquired by using this method. Application of simultaneous RNA/DNA extraction into routine can be very beneficial for forensic purposes.

In conclusion, microRNAs are very significant biomarkers in legal medicine and forensic sciences fields. In addition to conventional methods, they can be very supportive and information providing methodologies. Especially in the case of inadequate results which obtained with using conventional methods, accurate results may be obtained by using molecular based methods (for instance miRNA biomarkers) in the future.

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Conflict of interests
The authors declare that there is no conflict of interest in the study.

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References
21. YÖK National Theses Center. https://tez.yok.gov.tr/UlusalTezMerkezi/ access date 06.06.2022