NOFOR
NOVEL FORENSIC RESEARCH

DOI: 10.5455/NOFOR.2022.06.01

NOFOR 2022;1(1):10-3

REVIEW ARTICLE

RNA-Approached technology applications in forensic genetics

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Received 21 June 2022; Accepted 24 August 2022; Available online 20.12.2022

Available online at www.nofor.org

Abstract

Ribonucleic acid (RNA) is a nucleic acid which is structurally different from DNA. DNA is the most used and approved nucleic acid in routine applications in forensic caseworks. In this article, giving an information about which RNA types are chosen for which forensic applications is aimed. RNA technologies are developing in the concept of forensic genetics and they can be adapted into routine case works in the case of well conditions are supplied. Both coding and non-coding RNAs are investigated for forensic purposes and most examined ones are messenger RNAs, and microRNAs. There are some researches on circular RNAs and piwi-interacting RNAs but they are in low number when compared with first two RNA types. Forensic studies based on RNA technologies are body fluid identification, post mortem interval determination, determination of stain age, estimation of an individual's age and sex, identification of organ tissues, wound age estimation, and determination of drug abuse. Lastly, different RNA based technologies can be used in these studies and some of them are micro-array, Nano-String technology, real time PCR, end point PCR, high resolution melt (HRM) analysis and next generation sequencing (NGS) technology.

Keywords: RNA technologies, Messenger RNA, MicroRNA, Circular RNA, Piwi-interacting RNA, Forensic Genetics

Ribonucleic Acid (RNA)

Ribonucleic acid (RNA) is a nucleic acid which consist of phosphodiester bond linked nucleotide subunits. DNA and RNA have some differences structurally. DNA has deoxyribose sugar in its structure while RNA has ribose sugar. Furthermore, DNA and RNA contains adenine, guanine, and cytosine bases in common but fourth base in DNA is thymine while RNA has uracil. There are diverse types of RNAs and messenger RNAs (mRNA), ribosomal RNAs (rRNA), and transfer RNAs (tRNA) are the main types of RNAs which play important roles in protein synthesis [1]. The main types and the structure of RNA can be seen in Figure 1 below [2]. Messenger RNAs are in charge of protein coding while ribosomal RNAs catalyzes the synthesis of proteins and responsible from forming the ribosome's basic structure. Transfer RNAs work as adaptors between amino acids and mRNA in protein synthesis. In addition to these type of RNAs there are small nuclear RNAs (snRNAs, have role in nuclear processes) and small nucleolar RNAs (snoRNA, have role in the modification of rRNAs). Except these RNAs, there are other noncoding RNAs which play role in distinct processes in the cell [1] and some of them (especially microRNAs) are important in studies conducted in forensic sciences.

CITATION
Yukseloglu EH. RNA-Approached technology applications in forensic genetics. NOFOR. 2022;1(1):10-3. DOI: 10.5455/NOFOR.2022.06.01

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DOI: 10.5455/NOFOR.2022.06.01

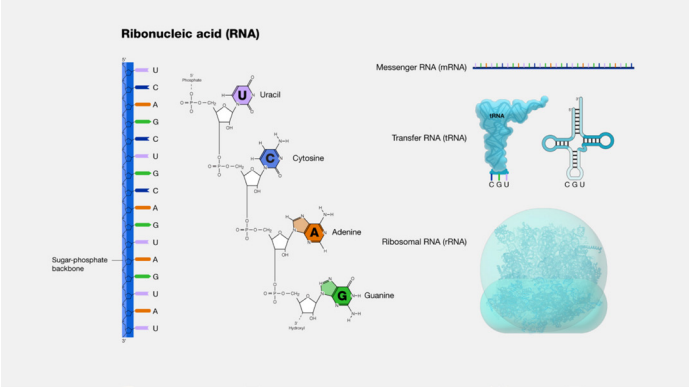

Figure 1. The structure of ribonucleic acid (RNA) and main types of RNA (mRNA, tRNA, rRNA) [2]

RNA in Forensic Genetics

Forensic genetics is an area which work multidisciplinary just as the remaining fields of forensic sciences. In other words, forensic genetics studies contribute to many research studies. Forensic sciences serve in order to prove the innocence of an individual and determine the real criminal for justice. Forensic genetics studies are especially important for the crime cases linked with biological evidences. With the development of technology, novel methods were brightened and molecular methods are started to interest researchers in this working field.

In the concept of forensic sciences, the most investigated RNA biomarkers are messenger RNAs (mRNAs) and microRNAs (miRNAs) according to literature. Studies which focus on mRNA in the forensic sciences field, started in 1994. In this study, Phang et al. reported the intact mRNA extraction from postmortem tissues by using RT-PCR [3]. Inclusion of miRNAs to forensic applications dated back to 2009. Hanson et al. conducted a study for identification of body fluids for forensic purposes by using miRNAs [4].

Messenger RNAs are coding RNA which transcribed from DNA and they carry the information needed for protein synthesis [1]. Secondly, microRNAs are small non-coding RNAs and they play a role in regulation of the genes [5]. When literature is viewed, body fluid identification studies are the most common studies performed by using RNA based technologies in forensic sciences. There are some limitations in conventional body fluid identifications methods. To illustrate, distinguishing the menstrual/venous blood in the sexual assault cases is a very challenging problem to perform by using conventional methods. These methods have some sensitivity and specificity problems in addition to time consumption. Because of these mentioned limitations, molecular based technologies are tried to apply in forensic purposes. Analysis of mRNA expressions have higher sensitivity and specificity when compared with conventional methods. In addition, mRNA analysis for body fluid identification has very important advantage as determination of more than one body fluid in one multiplex reaction [6]. In a study, researchers stated that endpoint PCR and real time PCR can be used as an alternative for conventional methods in order to determine body fluids [7]. In the same study, up to 2-year-old stains, mRNA stability in biological stains was shown.

As RNA based studies increase, comparison between biomarkers are becoming seen. Both mRNAs and miRNAs are tested for body fluid identification. When compared with miRNAs, mRNAs are thought less ideal for forensic samples which are found degraded frequently. Because of their amplification sizes and tissue specific features, interests on miRNAs are increasing [8]. In a study which simultaneous examination of miRNA and mRNA biomarkers in body fluids, study group stated that miRNA biomarkers are more advantageous when compared with mRNA biomarkers in degraded samples [9].

In addition to mRNAs and miRNAs, there are some regulatory non-coding RNAs examined for RNA based approaches in forensic sciences but they are low in number when compared with these two biomarkers. Circular RNAs (circRNAs) are pre-mRNA back-splicing products and they are found in high amounts in human cells. They show cell type-specific expressions and high stability [10]. It can be said that they have similar features like miRNA which is another type of non-coding RNA. In a study, inclusion of circRNAs in mRNA profiling was investigated and researchers concluded that this application improved the detection of mRNA biomarkers in bodily fluids for forensic purposes [10]. In another study, distinguish based on circRNA expression profiles of body fluids was performed and method was successful for distinction of venous blood, semen and saliva. In addition, method was not able to differentiate menstrual blood from vaginal secretion [11].

Another non-coding RNA tested for forensic applications is piwi-interacting RNAs (piRNA). They also show tissue-specific expressions and have short length. In a study, four piRNAs were examined in forensically relevant venous blood, menstrual blood, saliva and semen samples. One piRNA (piR-55521) was found in high expression levels for semen. Additionally, according to stability tests, they stated that piRNAs from dried samples could be detected both in laboratory and outdoor conditions for at least six months [12]. In another study, potential piRNAs for distinction of venous and menstrual blood were stated. In the same study, potential piRNAs for vaginal secretion and saliva distinction were also stated [13].

As seen in previously mentioned studies, more information about RNA technologies is obtained due to use of different biomarkers. Furthermore, with the comparison of diverse RNA biomarkers in different studies in the future, more accurate results may be obtained and more knowledge about human body may be collected in order to use for forensic purposes.

Because mRNA analysis may give information about the occurring events in a specific tissue types at a certain point of time, some researchers stated that mRNA analysis may be used for forensic purposes such as understanding the mechanisms leading to death or estimating the death time [14]. Determination

DOI: 10.5455/NOFOR.2022.06.01

of the exact time of death is quite important for forensic caseworks and it may provide significant information for solving the case. In a study, mathematical model was developed for PMI determination by using RNA degradation [15]. Researchers conclude that developed model may be used for complementary tools for conventional methods. Another study group investigated the human dental pulp samples for estimating PMI based on RNA degradation and they found promising results too [16]. In a systematic review, miRNAs were assessed as potential biomarkers for PMI estimation thanks to their tissue-specific expression nature and low molecular weight features [17]. Additional RNA based technology for forensic use is age determination of stains. For some specific cases such as sexual assault, determination of stain age may be quite important. In order to prove the relationship of a stain with crime, stain age determination method is quite important. In a study which blood and saliva stains were analyzed, results showed that mRNA can be extracted from biological stains up to two years old [18].

According to another study, distinct type of stains was analyzed and they found that global abundance of mRNA transcripts decreased with time. Additionally, no relationship between storage time and length of transcript was found [19]. RNAs are also be used for age prediction in the concept of forensic sciences. There are some age-related miRNAs and in a study, age prediction models for bloodstains were established [20]. In the same study, mean absolute error for males was found as 5.52 years while it was 7.46 years for females. In another research, two new isoforms of gamma hemoglobin mRNA were discovered and they found that these mRNA isoforms exhibit a gene expression pattern limited for newborns [21]. This information may be useful in newborn included forensic cases. When researches increase in the future, sex determination of individuals may be done by RNA technologies. To illustrate, two miRNAs (miR-130b and miR-18b) showed concentration dissimilarity among genders. They showed slightly higher concentration in male serum samples when compared with female serum samples [22]. Furthermore, mRNA content of some fat oxidation related genes showed difference between genders and they were found higher in females when compared with males [23]. When these type of studies improved, obtained data may be assessed more accurately and used for forensic purposes.

Another forensic genetic application of RNAs is identification of organ tissues. This application may be useful for especially in shooting-related crimes because of tissue scattering. To illustrate, crime tool can be identified or exact crime scene can be determined more accurately thanks to this method. By using mRNA profiling, an assay was developed in order to identify 10 distinct organ/tissue types [24]. In addition to mRNA based methods, miRNA based methods on organ tissue determination were also studied [25].

Drug abuse is a serious problem which includes in many forensic cases. Cocaine is one of the abused drugs and their behavioral effect was related with circRNA and miRNA interaction [26]. Additionally, non-coding RNAs (e.g. miRNAs) were stated as important biomarkers for addiction-related behaviors [27]. According to this information, it can be inferred that RNA based applications may be used for drug abuse determinations in the future. Lastly, wound age determination are examined with RNA methods. In a study, wound age was found related with seven genes and their mRNA expressions were analyzed for this information [28]. Wound age estimation is important in forensic sciences because they help to assess injury-crime relationship. The mRNA expression patterns of six genes were found related with the age of human dermal injury [29].

In all of these mentioned studies and the other RNA studies, different analyzing techniques can be chosen according to the needs of a study. Some of the RNA-based technologies used in forensic science studies can be listed as micro-array, Nano-String technology, real time PCR, end point PCR, and high resolution melt (HRM) analysis [30]. In addition to these technologies, next generation sequencing (NGS) is a useful method. NGS technology may be used for different forensic purposes and it provides simultaneous analyzes [31].

CONCLUSION

When compared to use of DNA profiling, RNA based approaches are less common in forensic science community. However, increasing number of studies and their results on this topic are promising for the future applications. With the application of novel technologies developed for scientific use, RNA based technologies can be applied in routine caseworks. As mentioned before, there are distinct types of RNAs. Both non-coding and coding RNAs are tested for forensic potentials but mRNAs and miRNAs are the most investigated biomarkers in the literature. In addition, circRNA and piRNA included studies in different topics were also mentioned in the article.

Applications of diverse RNAs in the field of forensic genetics are quite abundant and different studies are being conducted. Research topics can be listed as body fluid identification, post mortem interval determination, determination of stain age, estimation of an individual's age and sex, identification of organ tissues, wound age estimation, and determination of drug abuse.

With developing technologies, more than one techniques may be chosen by different research groups. In other words, there is no single method for analyzing RNAs. Different RNA based technologies can be used in RNA studies and some of them may be listed as micro-array, Nano-String technology, real time PCR, end point PCR, HRM analysis and NGS technology.

Acknowledgments:
This article was presented at the 3rd Turkish Forensic Sciences Congress held in Antalya on 2-5 June.

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

Financial Disclosure
The authors declare that they have received no financial support for the study.

DOI: 10.5455/NOFOR.2022.06.01

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