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mRNA profiling in forensic genetics I: Possibilities and limitations☆

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ABSTRACT

Molecular investigations gain increasing interest in forensic medicine. Examination of gene expression levels at the time point of death might have the power to become a complementing tool to the current methods for the determination of cause and circumstances of death. This includes pathophysiological conditions of disease and injury as well as the duration of agony or other premortem factors. Additionally, recent developments in forensic genetics revealed that tissue specific mRNAs can be used to determine the type of body fluid present in a crime scene stain.

Although RNA is known to be rather instable, RNA could be extracted in adequate quality from tissue samples collected during medico-legal autopsy. Nevertheless, working with human postmortem tissue means to deal with highly variable RNA integrities. This review aims to give a brief overview of the possible advantages of postmortem mRNA profiling and to shed further light into the limitations of this method arising from reduced RNA integrities.

1. Introduction

1.1. RNA as the intermediate template of protein biosynthesis

When James D. Watson and Francis Crick [1] described the three dimensional structure of DNA in 1953 this was the first idea on how genetic information is encoded and how it is transferred from one generation to the next. Now we know that the main precondition for the transfer of genetic information from one cell to its daughter cells as well as from one generation to the next is the high stability of genomic DNA [2,3]. Even though the structure of RNA is similar to DNA, its function is time limited resulting in relatively short half-lifes. One central assignment of RNA is the conversion of genetic information into proteins and the regulation of this process. During this, the two strands of DNA are separated and RNA is synthesised by RNA polymerases complementary to the coding strand. To ensure the possibility of regulation of gene expression it is crucial to avoid the accumulation of certain mRNAs. Thus, RNA needs to be degraded in the cell by omnipresent, highly reactive ribonucleases [4]. This is essential for the regulation of translation and therefore for the control of the amount of a gene product [5].

1.2. Different RNA families

By now, many different forms of RNA molecules are known which can be differentiated not only by their specific configuration and secondary structure but mainly by their function and half-life (Table 1). On the one hand there are RNAs, like *messenger* RNA (mRNA), *transfer* RNA (tRNA) and *ribosomal* RNA (rRNA) which are directly involved in the biosynthesis of proteins [6]. On the other hand there are numerous functional RNAs which for example are involved in the regulation of the gene expression process or which have a catalytic character, like ribozymes.

In the first step of protein biosynthesis, genetic information is transcribed into mRNA which is then transferred to the ribosome. The proportion of mRNA is only 3–5% of the total amount of RNA in the cell while the 28S, 18S and 5.8S rRNAs form the largest RNA family [7]. The rRNAs build the two ribosomal subunits. During translation they catalyse the binding of peptides. The tRNA is also involved in the process of translation and helps transferring the correct amino acids from the cytoplasm to the ribosomes [8]. Beside the initiation of transcription and translation the half-life of different RNA families is a crucial point in gene expression regulation. rRNA as well as tRNA remain stable for several days while different mRNAs show quite varying half-lives. There are rather instable transcripts which are degraded after minutes but also transcripts that remain stable for several days.

Now an additional group of relatively short RNA molecules is known. A number of small RNAs are also involved in the regulation of gene expression [9]. MicroRNAs (miRNAs) and anti-sense RNAs (asRNAs) are directly synthesised as single stranded molecules

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Table 1
Overview over different RNA families with their specific functions and half-lives.

Type of RNA	Abbreviation	Main function	Half-life
Messenger RNA	mRNA	Template for translation	Minutes to days
Transfer RNA	tRNA	Transport of amino acids	Days
Ribosomal RNA	rRNA	Ribosomal structure	Days
Small nuclear RNA	snRNA	mRNA processing	Days
Small interfering RNA	siRNA	Regulation of gene expression	Days to weeks
microRNA	miRNA	Regulation of gene expression	Days to weeks
PIWI interacting RNA	piRNA	Regulation of gene expression	Days to weeks
Anti-sense RNA	asRNA	Regulation of gene expression	Days to weeks
Riboswitches	–	Regulation of gene expression	Days to weeks
Ribozymes	–	Enzymatic function	Days

while small interfering RNAs (siRNAs) are cut from double stranded pre molecules with the help of dicer [10]. Mature miRNAs and siRNAs usually have a length of only 21–26 nucleotides. Similar to asRNAs, they are involved in post-transcriptional mRNA degradation and the control of translation activities [11,12]. The expression of miRNAs is mainly tissue specific [13] and they act as marker for post-transcriptional gene silencing: miRNA binds to the 3′-untranslated region (3′-UTR) of the mRNAs which then are either destroyed by nucleases or their translation is blocked [14]. Current developments in biochemistry revealed that the expression of up to 30% of all genes is regulated by miRNAs [8].

A rather specific group of small RNAs is called PIWI interacting RNAs (piRNAs). They are involved in gene silencing of transposons during spermatogenesis [15,16]. To fulfil the function of gene regulation, the family of small RNAs needs to show a comparably long half-life with up to two weeks.

A further family of relatively short functional RNAs comprise small nuclear RNAs (snRNA) which usually have a length between 90 and 190 nucleotides. They are responsible for processing of pre mRNA into mature mRNA within the splicosome [17].

2. Possible applications in forensic genetics

Many cellular decisions, including survival, growth and differentiation are regulated by specific gene expression patterns [18]. Thus, the possibility to quantify gene expression levels of specific genes currently gains increasing importance in a wide range of scientific disciplines.

mRNA as the intermediate template in protein synthesis is a target for the analysis of gene expression patterns because it reveals the activities of genes within a certain tissue type at a certain point of time. Of course, the question of correlations between changes in the normal expression pattern and pathological changes of the tissue arises immediately. From a medico-legal point of view, the analysis of gene expression patterns might give hints on pathological states [19] or the circumstances leading to death [20]. Some authors hope to use specific mRNA degradation patterns as a new tool for the accurate determination of the time since death [21].

2.1. Identification of body fluids

Blood and saliva are considered the most common types of body fluid that form a common source for genetic information used in forensic science. But also ejaculate, vaginal secretion, lacrimal fluid and sweat are common subjects. The determination of the type of body fluid is important to insure the correct handling of samples, e.g. in cases of mixtures from different sources like ejaculate and vaginal secretion [22]. Current tests for the identification of body fluids use chemo luminescence and the detection of specific proteins (e.g. [23]). Due to the fact that gene expression patterns are tissue specific, a determination of the type of body fluid based on mRNA profiling may be possible. First experiments show that RNA can be isolated in suitable quality and quantity from menstrual blood [24,25], blood, saliva and vaginal secretion [26–28].

Recently, a number of articles concentrating on the practical considerations connected to mRNA profiling using crime scene stains were published by several authors. These works address the challenge of isolating mRNA and DNA in suitable quality and quantity from limited amounts of stains [29,21] as well as the validation of methods to analyse certain transcripts, e.g. by end point PCR or real time PCR [23,30] and suitable sets of stable mRNA markers [31,28].

One problem when working with crime scene stains is that due to degradation, false negative results are possible. Additionally, many transcripts are not completely tissue specific but may show high expression levels in one body fluid and very low expression levels in another. Thus, a qualitative analysis, e.g. using end point PCR, might not be appropriate but rather quantitative analyses are suitable. Nussbaumer et al. [32] showed that kalikrein 3 (KLK) transcripts are detectable in ejaculate but not in blood, saliva and vaginal secretion. Thus, in this case analysis via end point PCR is sufficient. This group also showed that other transcripts, like hemoglobin-alpha-1 (HBA) and mucein (MUC) show high expression levels in ejaculate and vaginal secretion, respectively, but are also detectable in other body fluids. This condition requires a quantitative approach. In quantitative analyses, the validation of a suitable normalisation strategy plays a central role [33]. Without normalisation, analyses might lead to false negative or false positive results. But also in cases of qualitative analyses, it seems reasonable to include a quality marker to exclude the risk of false negative results. A useful quality marker might be the transcript of a low expressed gene which degrades similar or even faster than the gene of interest. Another approach for this problem was described by Juusola and Ballantyne [27] by using not one marker for each body fluid but a set of three markers.

Since gene expression patterns are tissue specific it has to be expected that also the presence of certain regulatory elements, like microRNAs, are highly tissue dependent. Due to their high stability and comparably long half-lives, microRNAs gained increasing interest for their use in body fluid identification recently [34,35].

2.2. Molecular determination of the cause and circumstances of death

The composition of different RNA transcripts within the cells of a tissue, the RNA pool, is not considered stable, but changes according to the specific needs of the cell in response to external conditions. Thus, each event, may it be a molecular event or some external impact influencing the whole organism, leaves a molecular mark in terms of shifted proportions of different