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Using mRNA biomarkers to distinguish skin cells from other contact DNA traces for forensic purposes

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Within the discipline of forensic science, being able to pinpoint the DNA profile of a possible suspect is extremely vital to solving a crime. If the understanding of the cellular origin of the DNA evidence is known, eliminating suspects becomes more straightforward. Specifically DNA evidence of epithelial origin could mean all the difference in categorizing suspects. In the past, there have not been methods for determining epithelial cells from other biological evidence. Recent studies have been conducted to find mRNA biomarkers that when utilized in conjunction with reference biomarkers can give insight into the cellular origin of biological evidence discovered at a crime scene. mRNA biomarkers, CDSN, LOR, KRT9 and LCE1C seem to be the key to doing precisely this. A multiplex assay that utilizes CDSN and LOR along with other RNA biomarkers for determining cellular origins has been developed. If these findings can be refined for common utilization within the forensic science discipline, this can greatly reduce the number of suspects during any given criminal case as well as give insight into what took place during the criminal act. The next step would be refinement and elucidating these findings to criminal justice professionals.

Introduction

Forensic science has been around for many centuries, but it has not always been a precise science and allowed for much conjecture. During the late 1800s, body measurements were utilized to identify criminals, but this was not a precise testing method. In the early 1900s, fingerprinting became the most widely utilized technique in determining suspects. Unfortunately, fingerprints are not always found at a crime scene. It is only within the last few decades that certain significant advances have been made. One of these advances has been the utilization of DNA evidence in determining suspects. DNA evidence began to be utilized in the late 1980s to solve crimes, and since that time, DNA evidence has been the foremost method for doing so. Crime scene reconstruction is another important tool that is utilized when attempting to solve a crime. Understanding which type of biological trace is uncovered can be highly important. Numerous tests can be utilized to identify cellular origin of biological traces as criminal evidence. These types of presumptive, protein-based tests are mostly utilized to detect traces of saliva, blood or semen. An issue with these testing techniques is that they frequently have a low sensitivity and occasionally produce false positives. This is attributed to presumptive tests being utilized to exclude or determine that trace evidence is possibly a specific biological substance. This is why research into new techniques that are more sensitive and accurate has been of great importance.

Recently, forensic scientists have begun using RNA biomarkers, specifically mRNA biomarkers, in the solving of crimes. A biomarker is a molecule that allows for recognition and isolation of a specific cell type. mRNA has been found to be the best biomarker because it is specific for each cell type. Most importantly, mRNA is utilized to distinguish between various types of bodily fluids and tissues found within a crime scene. The same DNA is established in all cell types making this unavailable for utilization when distinguishing between cell types. Proteins are different in each cell for the reason that they have different functions based on cell types. This renders the utilization of mRNAs as an

appropriate measure for determining cell type for the reason that they encode the proteins made for each specific cell type. Within the last couple of years, the examination of mRNA biomarkers for 'touch' or 'contact' DNA (DNA transferred by physical touch by a donor) has become imperative. For instance, being able to distinguish between donor buccal epithelial cells from saliva and donor palmar epithelial cells can be significant [1]. It can then be determined if a suspect had only talked to the victim or touched the victim. This provides a much more precise picture of what may have taken place during the act of the crime.

Skin mRNA biomarkers and biomarker multiplex

Recent research has uncovered several mRNA transcripts that could possibly be utilized as biomarkers for determining if biological evidence is from contact DNA. Because epithelial DNA can be found within the cornified layers that are shed easily, most mRNA biomarkers uncovered for determination were found in the cornified layers [2]. In the study conducted by Visser et al., ten candidate skin genes were selected to test for high expression [3]. When combined with the reference biomarker ACTB, transcripts CDSN, LOR and KRT9 were revealed to have the highest expression, making them promising biomarkers [3]. Corneodesmosin (CDSN) encodes a protein important for the integrity of the epidermal barrier. LOR encodes for the protein loricrin which is important for the keratinocyte cell envelope. Keratin 9 is encoded by the gene KRT9, and is exhibited in epidermal cells of the palms and soles of the feet.

In the study conducted by Hanson et al., 103 candidate genes were chosen for testing [1]. Of the 103 genes, five were selected for further testing [1]. The biomarker with the most potential was discovered to be LCE1C [1]. LCE1C stands for late cornified envelope 1C, and its function is to produce precursors for the cornified envelope of the stratum corneum. Even though Hanson et al. created two multiplexes of skin biomarkers from their study, LCE1C was, nevertheless, considered the best candidate genes from those tested [1]. The amount of RNA found within a cell is between 20-30 pg. Hanson et al. were able to utilize samples with 25pg or less of RNA total [1]. This means that the sensitivity of an assay utilizing the LCE1C biomarker is approximately that of a single cell or smaller [1].

Lindenbergh et al. developed a multiplex assay using 19 mRNA biomarkers for six cellular types [4]. The six cellular types are blood (3 biomarkers), saliva (2 biomarkers), semen (2 biomarkers), skin (2 biomarkers), menstrual secretions (2 biomarkers) and vaginal mucosa (2 biomarkers) [4]. Three biomarkers were mucosa markers utilized in conjunction with the biomarkers from saliva, menstrual secretion, and vaginal mucosa [4]. Another three biomarkers were considered housekeeping biomarkers that could be utilized in conjunction with any of the six cellular types [4]. CDSN and LOR are two skin biomarkers that were utilized in the development of this multiplex assay [4]. LCE1C was not a biomarker that was utilized during the development of the multiplex assay for the reason that both studies were published with in the same journal issue. Most biomarkers were found to be expressed in old samples ranging from 4 to 28 years [4]. This can be very useful in reconstructing a crime scene and configuring a timeframe.

Discussion

Even with the many advances in the discipline of forensic science, reconstruction of the crime scene and determination of the manner of the crime has not always been straightforward. In the past when DNA was found at a crime scene, forensic scientists had few methods available to determine the cellular origin of that DNA evidence. None of these methods could identify DNA evidence transferred by skin contact. Knowing beyond a reasonable doubt that DNA evidence was transferred by skin contact of a suspect and not from another cellular origin can lead to a much more sophisticated forensic science discipline. This also leads to fewer assumptions as to who may or may not be suspects. Recent research within the last couple of years had made much progress in moving towards this goal. The findings for the epithelial biomarkers may not seem significantly sensitive or specific on their own. However, when these biomarkers are utilized in conjunction with the multiplex assay of mRNA biomarkers, the sensitivity and specificity of the biomarkers is considerably higher [4].

It is extremely important for data to be sensitive and specific when solving crimes so there are no false positives or negatives. With the recent dilemma that has arisen due to DNA evidence not being interpreted correctly, introducing new techniques of identifying suspects has become increasingly difficult. These can lead to lawsuits, appeals, or the release of true criminal suspects. One such instance is the court case concerning the murder of Peter Hoe in the United Kingdom [5]. Terence and David Reed were convicted of the murder for the reason that DNA matching the both of them was found on the knife discovered by the body of the victim [5]. An appeal was made on the basis that not knowing how the DNA was transferred to the knife and what type of epithelial cells were transferred [5]. It was also not known whether the transfer was by primary, secondary or tertiary transfer [5]. Time can also be a factor that causes problems with any biological sample found. The longer the time between discovery and testing, the likelihood that the sample can degrade before accurate results can be developed. Even though the 19 biomarker multiplex does seem to produce profiles even after many years have

passed, more research should be conducted to refine these findings and to produce the most sensitive and specific biomarkers for utilization in the multiplex assay. Following this, the next step would be explaining the finding of RNA-based forensic tissue identification and their interpretations to criminal justice professionals [4].

References

[1] Hanson, E., C. Haas, R. Jucker, and J. Ballantyne. 2012. Specific and sensitive mRNA biomarkers for the identification of skin in 'touch DNA' evidence. Forensic Science International: Genetics **6**: 548-558.

[2] Kita, T., H. Yamaguchi, M. Yokoyama, T. Tanaka, Noriyuki Tanaka. 2008. Morphological study of fragmented DNA on touched objects. Forensic Science International: Genetics **3**: 32-36

[3] Visser, M., D. Zubakov, K.N. Ballantyne, and M. Kayser. 2011. mRNA-based skin identification for forensic applications. International Journal of Legal Medicine **125**: 253-263.

[4] Lindenbergh, A., M. de Pagter, G. Ramdayal, M. Visser, D. Zubakov, M. Kayser, and T. Sijen. 2012. A multiplex (m)RNA-profiling system for the forensic identification of body fluids and contract traces. Forensic Science International: Genetics **6**: 565-577.

[5] R.a.G. R v. Reed [Nos: 2007/04708/B3, 2007/04710/B3, 2007/04800/D4], High Court Justice Court of Appeal (Criminal Division), Royal Courts of Jusic, Strand, London, WC2A, 2LL, 2009.

