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Beyond DNA: Epigenetics and Proteomics in Forensic Science

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Abstract
The use of genetic evidence in criminal cases is well established and has improved the public opinion and credibility of forensic science. However, several shortcomings associated with current genetic profiling techniques exist. Scientific research aimed at increasing the overall knowledge and understanding of biological factors will lead to the development of methods capable of improving the discriminating power of DNA evidence, overcoming limitations associated with DNA evidence, or complementing current methods of DNA profiling. Increased research in the fields of epigenetics and proteomics are particularly promising and relevant to forensic science. Research suggests that epigenetic biomarkers can be used to approximate the age of biological sample donors, differentiate between DNA of monozygotic twins, distinguish between natural and synthesized DNA, and identify body fluid sources from forensic material. Proteomic research studies indicate that mass spectrometry can be used to identify biological matrices and tissue sources from forensic biological samples without compromising DNA evidence. The demand for improved forensic techniques necessitates further research into these fields and, specifically, how the associated methods can be used in forensic science.

Keywords
epigenetics, proteomics, DNA, forensic evidence

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VOLUME IV • 2016

Introduction

The introduction of genetic material as evidence to forensic science applications has undeniably had an immense impact on the field. DNA can serve as infallible evidence in criminal cases, and is considered impervious to subjectivity and bias; for this reason, DNA evidence is often regarded as the paragon of forensic evidence. As with any science, increased research will result in methodologies that improve current techniques as well as new and innovative methods to aid in criminal investigations. Discovery of the human genome and its implications introduced possibilities initially outside the scope of many scientists' imaginations. Nevertheless, further research has shown that the genome is not entirely predictive of phenotype: many other biological factors are involved in gene expression. Just as an increased understanding of the human genome led to the development of influential analytical forensic applications, current and future research expanding on the knowledge of biological factors will lead to the development of more reliable and effective methods in forensic science that can be used in tandem with DNA profiling or to increase the efficacy of DNA profiling.

Current Techniques in Forensic DNA Analysis

DNA is a biomolecule containing genetic material that is found in all biological cells (except mature erythrocytes) and is unique to all individuals (Alberts, Johnson, Lewis, & et al., 2002). DNA analyses are used in forensic science to identify murder victims, potential suspects, or victims of mass disasters; in addition, DNA evidence can be used to exonerate innocent individuals and determine paternity. Specifically, short tandem repeat (STR) regions of DNA are used for forensic DNA

identification purposes (Norrfard, 2008). The number of repeated sequences in each STR region varies drastically between individuals, and comparing multiple STR regions increases the discrimination power of DNA evidence (Norrfard, 2008). The introduction of polymerase chain reaction (PCR) as a method of amplifying DNA further revolutionized forensic science, as this method allows for DNA to be obtained from sample sizes previously deemed insignificant (Norrfard, 2008).

Epigenetics

While the implications associated with genetic evidence are robust, the fields of epigenetics and proteomics are growing and the associated potential applications are forthcoming. Just as genetics is the study of the genome, epigenetics is the study of the epigenome. The epigenome refers to external chemical changes to the DNA or associated histone proteins that do not alter the genetic profile but result in a heritable change in phenotype (Alberts et al., 2002; Goldberg, Allis, & Bernstein, 2007). These chemical modifications can affect how genes are expressed without altering the genetic material and effectively turn genes on or off (Goldberg et al., 2007). While epigenetic modifications are heritable, they are also reversible (unlike genetic mutations) and can include DNA methylation and histone modification (Goldberg et al., 2007). DNA methylation, the covalent addition of a methyl group to a DNA nucleotide, has been extensively studied and has become particularly relevant to forensic science (Grskovic, Zrnec, Vickovic, Popovic, & Mrsic, 2013).

Proteomics

Analogously, the study of the proteome is denominated proteomics. The proteome describes all proteins that a genome expresses; while some genes may be encoded in the genome,

they may or may not be expressed as proteins and thus, may or may not be included in the proteome (Berg, Tymoczko, & Stryer, 2002). Alternative RNA splicing is a process that can result in one gene that codes for multiple proteins; this happens when the messenger RNA (mRNA) is produced and different combinations of introns can be removed—termed RNA splicing (Berg et al., 2002). Alternative splicing that occurs causes a range of proteins to be produced based on the associated series of exon compositions that can occur after RNA splicing. Additionally, post-translational modifications to proteins can also result in increased protein variety (Mishra, 2010). Post-translational modifications of proteins occur when a new functional group is covalently attached to a protein after translation of the protein from mRNA is complete (Mishra, 2010). By changing the function of proteins in the cell when a new functional group is added to the protein through covalent bonding, protein diversity is increased on behalf of these post-translational modifications. The genome and the epigenome are two of several factors influencing the proteome; additionally, modifications to histone proteins can affect histone-DNA interactions and thus influence gene expression, altering the epigenome (Alberts et al., 2002).

Shortcomings of Current Techniques

Several challenges associated with forensic STR profiling exist. Specifically, one such issue arises when available bodily fluid evidence is sparse. Efficient examination of samples at the crime scene using non-destructive methods is crucial to minimize the time and cost associated with investigations, and to preserve any DNA evidence that can be analyzed at a later time (Lee et al., 2012). However, when sample sizes are too limited to undergo the multiple tests required for identification or contain a

mixture of fluid types or fluid contributors, current methods may fall short (Virkler & Lednev, 2009). Limited sample quantity is frequently a challenge in DNA analyses. However, the destructive nature of many biochemical tests remains the principal disadvantage; since many tests are specific for only one marker, there are often multiple tests involved in identifying sample sources (Virkler & Lednev, 2009). The need for multiple tests (cascade testing) is often not agreeable with the available sample size and can compromise the potential for later DNA testing; furthermore, cascade testing is neither cost nor time effective.

It has also been suggested that those with some background in molecular biology, access to appropriate instrumentation, and knowledge of standard techniques in the field would be capable of synthesizing DNA with a specific genetic profile *in vitro*. Current forensic techniques are incapable of distinguishing between DNA that is biologically generated and that which is chemically synthesized, introducing the potential for DNA evidence fabrication (Frumkin, Wasserstrom, Davidson, & Grafit, 2010). Such contamination could be particularly damning considering the current general confidence in DNA evidence and simultaneous ignorance of the biology. Unfortunately, while advances in scientific research can promote advanced forensic science practices, they can also inform and inspire creative, well-informed criminals.

Forensic Applications

Considering both the shortcomings associated with DNA evidence and the general public's simultaneous demand for and confidence in DNA evidence, it is crucial that forensic science techniques continue to improve. Epigenetic and proteomic

research appears promising and the associated new scientific methods being developed could potentially overcome current limitations or reinforce the validity of DNA evidence.

Epigenetics

While epigenetic and proteomic techniques could potentially serve to support DNA evidence, research suggests that scientific methods assessing epigenetic or proteomic markers could also be used independently when DNA evidence is insufficient. As epigenetic changes can occur following exposure to certain chemicals, epigenetic analyses may be used in toxicological studies in the future. While further study is required to increase understanding of these effects, it is known that epigenetic mechanisms help regulate biological responses to toxicants (Williams, Mirbahai, & Chipman, 2014). Specific types of compounds appear to cause particular, recognizable changes to the epigenome in mice and in certain fish (Chappell et al., 2014; Williams et al., 2014).

Additionally, epigenetic modifications at specific genomic sites have been shown to be the most forensically promising biomarker associated with human aging (Weidner et al., 2014). Epigenetic modifications (DNA methylation, specifically) could serve as simple biomarkers allowing scientists to provide an approximate age of the sample donor. While this may seem insignificant, such information could be of great assistance to investigators when attempting to identify the source of bodily fluids.

The most obvious and cited disadvantage of current DNA profiling methods is the inability to differentiate between monozygotic twins, who share identical DNA. While genomic DNA of monozygotic twins is identical, evidence suggests that monozygotic twins are epigenetically distinct (Zhang et al.,

2015). Therefore, DNA methylation patterns could potentially be used to differentiate between samples when faced with a forensic conundrum of this type.

Furthermore, epigenetic analyses would allow investigators to distinguish between natural and synthesized DNA, as all synthetic DNA is unmethylated (Frumkin et al., 2010). Such an analysis could be invoked when fabrication of DNA evidence is suggested, bolstering the reliability of DNA evidence.

DNA methylation can also be used to identify body fluid sources and current methods are compatible with DNA STR profiling techniques (Romeika & Yan, 2013). Recent studies show that the detection of specific DNA methylation markers allows for the identification of venous blood, menstrual blood, saliva, vaginal fluid, and sperm from forensic material, including mixed samples (Forat et al., 2016).

Proteomics

Similarly to epigenetic profiles, proteomic profiles vary within individuals based on biological matrices and tissue types – an obviously advantageous characteristic as genetic profiles are the same in all cells of an individual (Dammeier et al., 2016; Forat et al., 2016; Van Steendam, De Ceuleneer, Dhaenens, Van Hoofstat, & Deforce, 2013). Protein primary structure (amino acid sequence) is extremely stable and can be determined via mass spectrometry (MS) even after protein degradation and after long periods of time (Van Steendam et al., 2013). MS has been shown to be an extremely sensitive and accurate technique and is often superior to immunoassays, due to response variability (Pitt, 2009).

While sequence determination of proteins through MS analyses is not yet possible, using enzymatic or chemical

digestion in combination with MS can allow for database searching in order to identify proteins (Trauger, Webb, & Siuzdak, 2002). For example, enzymes such as trypsin or chymotrypsin cleave proteins at specific amino acid residue sites. By subjecting a protein to digestion in this manner and then obtaining mass data of the peptide products, researchers are able to then input the associated masses into an appropriate database that would then use searching algorithms to identify the whole protein based on individual fragment sizes and known cleavage sites of the respective enzyme. Post-translational modifications often create molecular weight changes in a predictable manner, therefore, MS can also be used to identify particular post-translational modifications in proteins (Trauger et al., 2002).

MS techniques have been shown to be effective in identifying forensically relevant biological matrices, including blood, saliva, semen, and vaginal fluid (Van Steendam et al., 2013). Interestingly, analysis in this manner is performed on a byproduct produced during DNA analyses that is typically discarded, so this testing would not interfere or inhibit the potential for DNA profiling (Van Steendam et al., 2013). Another advantage to this method is the use of one single test to identify a cellular matrix, as opposed to typical cascade testing. It has been shown that this MS-based approach is capable of identifying both pure and mixed biological matrices of different species without compromising DNA evidence (Van Steendam et al., 2013).

Proteomic analyses have recently been shown effective for identifying specific tissue types as the source of blood samples. A recent study used proteomic MS analyses to identify the tissue sources from biological material on the surface of a projectile (Dammeier et al., 2016). Such capabilities could

expedite forensic reconstructions, and help confirm the source of lethal injury if it is in question. While DNA profiling of blood samples is routine, it can often be challenging to determine such specific—and often forensically significant—details.

Current research goals aim to develop methods to improve the throughput, specificity, and sensitivity of MS techniques (Grebe & Singh, 2011). However, the use of MS for peptide and protein studies has developed into a critical tool in analytical biochemical analyses. The MS capability to identify and quantify proteins will have an increasing impact on molecular and forensic biology. Recent developments have improved the quantitative power of MS and developing research shows promise for improving MS techniques for proteomics.

Conclusion

As scientific research advances, so will the discriminating power of forensic techniques. Additionally, new techniques will continue to be developed in order to approach forensic problems that are not adequately addressed by current methods. For this reason, it is important to acknowledge the need for increased resources devoted to forensic science research.

Future directions in epigenetic research suggest that DNA methylation may become a prominent tool for forensic scientists. While proteomic analyses do not appear as imminent in the field as epigenetics, it is quite likely that future research may render proteomic analyses valuable to criminal investigations. Individual epigenetic and proteomic profiles are distinct and can be specific to tissue, age, and external factors such as stress or diet (Rothstein, Cai, & Marchant, 2009). Thus, epigenetic and proteomic research may prove to facilitate the next revolutionary scientific advancements in forensic science applications.

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