B117 A Proteomic Analysis of Epidermal Squamous Corneocytes

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Learning Overview: After attending this presentation, attendees will learn about proteomics and how it can be used as a potential means of human identification. Attendees will also learn how bottom up discovery allows for inference of genotypes.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating a potential new tool for consideration when DNA alone is insufficient, as it often is in touch evidence and fingerprints.

The DNA found in biological samples collected as evidence is not always a sufficient source of genetic information. An additional application of proteomics can allow for the expansion of identifying information available to the forensic investigators. Proteins can be an even better source at times because of its greater stability and abundance. Protein-based human identification experimentation begun with studies on peptides generated from hair. Recent advancements in instrumentation and bioinformatic tools have added further to this approach. The experimental focus is on the detection of genetically-variant peptides (GVPs) that result from changes in an amino acid sequence. Further, these single amino acid modifications are seen when variation exists in protein encoding genes as non-synonymous single nucleotide polymorphisms (nsSNPs). Thus, identification of single amino acid polymorphisms (SAPs) allows for DNA content to be inferred.

To demonstrate the utility of proteomics in forensic science, the protein population of skin cells was thoroughly examined and compared with respective exome data. This is significant because DNA found in touch evidence and fingerprints is often fragmented or degraded. A method has been developed to detect peptides of epidermal squamous corneocytes (ESCs) to catalog all the nsSNPs expressed in the specific proteome. To achieve this goal, exome data were filtered for missense SNPs to use as a comparison. First, corneocytes were collected from the same general area with the use of five dermal patches. Afterward, these cells were extracted with sodium dodecyl sulfate and washed with sodium dodecanoate, before dithioerythritol reduction, iodoacetamide alkylation, and trypsin digestion were performed. The digested proteins then underwent downstream mass spectrometric analysis. Since information on genetic variation can flow from proteomic to genomic data sources and vice versa, both bottom up and top-down GVP discovery techniques were used separately. The generated datasets were aligned with proteomic databases for peptide and protein identification and analyzed in comparison with exomes for the presence and confirmation of GVPs.

From an initial analysis of four subjects, 84 GVPs have been characterized and validated by DNA sequence. The cumulative number of GVPs identified per individual ranged from 24 to 32. Most notably, 2 rare GVPs were found (MAF < 0.0001). Without incorporating these, the application of the product rule led to a random match probability (RMP) of 2.36×10^{-06} in the European population. However, with the inclusion of the rare GVPs, the RMP decreased to 1.63×10^{-07}. Out of 336 inferences made, 10 were false positives. Recently, three more subjects have been evaluated. These datasets have produced 22 different GVP candidates, potentially bringing the total up to 106. The additional GVPs found could be the result of examining more individuals of a differing population. For each of the new individuals, the cumulative number of GVPs noted ranged from 26 to 31. Upon validation, the RMP is anticipated to decrease even further. Overall, the data depicts that proteomic processing of epidermal squamous corneocytes can lead to additional genomic content that is not always readily available at a crime scene. Going forward, a standard set of GVPs isolated and analyzed by this method needs to be determined. Thus far, GVPs corresponding to 13 genetic nsSNP loci have appeared in each subject. The reproducible results illustrate the potential use of this technique in the forensic science field. In the future, other tissue types can be assessed in the same manner.

Proteomics, Epidermal Squamous Corneocytes, Genetically Variant Peptides