Review Article

Forens(om)ic medicine: omics in forensic medicine

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ABSTRACT

The branch of science informally known as ‘omics’ are various branches in biology whose names end in the suffix omics such as proteomics, metabolomics, genomics, transcriptomics. The studies regarding the role of omics in various branches of medical field have become an upcoming area and its rising trend has been observed in the last decade. Omics has very recently emerged as an uprising field in forensic medicine also which is already known for its great participation with other branches like forensic toxicology, biochemistry, psychiatry, genetics. The use of state-of-the-art omics technologies has been explored in forensic medicine and sciences in establishing post mortem interval, drugs of abuse, intoxication and cause of death. The various forensic studies presently are aiming omics for future prospective. An elementary attempt has been made in this review article to briefly present the role and advantages of omics in forensic medicine. We, hereby, propose to call it forensomic medicine, to draw attention of the forensic professionals to this novel branch of science in order to encourage new studies to be used in medico legal applications.

Keywords: Omics, Post mortem interval, Biomarkers, Toxicology, Drugs of abuse, Spectroscopy

INTRODUCTION

Omics is the newly coined term for the collection of an organism’s omic information, which includes the genome itself (genomic), transcription products (transcriptomic), protein products (proteomic) and metabolic products (metabolomic).¹ Omics aims at the combined characterization and quantification of pools of biological molecules like proteins, metabolites, genomes, transcriptomes that translate into the structure, function and dynamics of an organism. The recent developments in technology have made omics based studies not only faster but cost effective, very informative and an upcoming alternative to conventional techniques to conduct a more efficient forensic investigation. The inter-relationship of various omics with each other and their applications in forensic medicine has been illustrated in Figure 1 and are discussed in the following sections.

Human blood, urine, hair, nail, feces, aqueous humor, body tissue can be used as samples for omics analysis in forensic medicine. The availability of the minutest amount of sample can help in extracting the information in a timely manner by using omics based technologies that would otherwise have taken months to years to obtain.²

GENOMICS

Genomics is the study of whole genomes of organisms and integrate elements from genetics. A human genome contains over 3 billion base pairs and over 30,000 human protein coding genes. Difference of genetic material between two people is approximately 0.1% which makes each of the human being different from the other.³ Various genomic based techniques used for omics analysis are real time polymerase chain reaction (RT-
PCR), microarray, next generation sequencing, matrix assisted laser desorption ionization-time of mass spectrometry (MALDI-MS).

**APPLICATIONS OF GENOMICS IN FORENSIC MEDICINE**

**Detection of the biological specimens**

Detection of DNA has contributed to match a suspect to a scene of crime through identification of body fluids. DNA fingerprinting based on variability and polymorphism in the DNA is already in use since early 1990s to establish a link between biological evidence and a suspect in a criminal investigation as well as to track down blood relations especially in paternity disputes. The presence of these fluids, or overall cavity has been protected from being guilty by the environmental factors and the role of bacterial nucleases.

Various studies have obvious limitations such as the influence of genomic approaches which might enumerate DNA degradation both in early and late post mortem interval using genomic approaches. Different microbes played specific roles during each stage of decomposition process. The microbes that colonized the cadaver and the rate of their activity were determined by the cadaver itself and its surrounding environmental conditions. A significant correlation had been observed for overall microbiome as the decomposition progressed by using novel throughput metagenomic sequencing and statistical model which had remarkable prospects in finding out DNA degradation studies have, however, shown that the use of DNA degradation to estimate PMIs, to date were still unpromising to standardize keeping in view the influence of ante and post mortem factors on DNA degradation.

**Intoxication and abuse of drugs**

Pharmacogenomics and toxicogenomics methods may find wider applications in forensic medicine in years to come. It may protect someone from being convicted as was evident from a published study of nine years old boy who had multiple psychiatric disorders and was on medications such as methylphenidate, clonidine and fluoxetine. He developed GI toxicity, disorientation, incoordination and seizures after ten months of medication and died of cardiac arrest subsequent to status epilepticus. The postmortem toxicological report of deceased showed several fold higher fluoxetine and norfluoxetine concentration. The parents of the deceased were consequently investigated for possible fluoxetine intoxication. However, pharmacogenomics and toxicogenomics studies of deceased showed that boy had a deficient p450 CYP2D metabolizer genotype which resulted in accumulation of fluoxetine. The child’s parents subsequently were acquitted of all charges. It led to the introduction of a new term personalized justice which supplemented a combination of translational and personalized medicine as in the above case, wherein the child’s parents were protected from being guilty by the use of pharmacogenomics and toxicogenomics methods.

**Diagnosis of disease and cause of death**

In forensic medicine, genomics-based approach also aimed to detect the genomic risk of the individual to establish the basis, causes and mechanisms of death so as to provide evidence based unbiased approach to death assessment. It also had an added benefit of providing feedback to the blood relatives of the deceased for similar genomic risk and direct them to a clinician for appropriate prevention of the onset of a particular disease. The application of genomics was thus of importance to find the cause and mechanism resulting in death at molecular-level which was commonly defined as molecular autopsy.

The conventional autopsy may be substantiated by various genomic approaches which might enumerate detection of cause and mechanism of death. A study based on molecular autopsy of 49 sudden unexpected death (SUD) cases observed a pathogenic cardiac channel
mutation in 35% of SUD cases which involved the mutation in the gene RyR2 (cardiac ryanodine receptor2) in one of every seven cases of SUD. Cardiac molecular autopsy, therefore, had the potential to provide a physio-pathological basis for SUD.

Figure 1: Diagrammatic representation of inter-relationship between different omics along with their applications and various analytical techniques used in forensic medicine.
TRANSCRIPTOMICS

Transcriptomics is a comprehensive analysis of whole sets of RNA transcripts produced by entire genome for a particular cell, tissue, organ or whole organism. Transcriptomics encompasses everything relating to RNAs which can be studied using novel technologies such as microarray analysis which quantify a set of predetermined sequence; RNA-Seq which uses next generation high-throughput sequencing to record all transcripts and PCR as common analytical methods.14

APPLICATION OF TRANSCRIPTOMICS IN FORENSIC MEDICINE

PMI

The analysis of mRNA provided quantitative evidence to know cell or tissue viability during the process of local necrosis or cell death. The study of mRNA expression of basic fibroblast growth factor (bFGF) to detect the age of wound in various wound locations of mice concluded that the time-dependent expression of mRNA of this growth factor in skin and cerebrum was useful for estimating the age of wound.15 It had been observed by the transcriptomics studies that DNA synthesis induced during life continues after death and can be used in determining the age of a wound post mortem. For example, the synthesis rates of RNA and DNA continued after death in intravital and post mortem biopsies from the site of injury caused by experimental wounds to rats’ both ears.16,17

Diagnosis of disease and cause of death

The transcriptomics analysis in specimens obtained in early postmortem period showed encouraging outcome for future prospective in disease diagnosis and cause of death. The investigation of transcriptome of brain samples of various schizophrenic, bipolar and depressive patients by using RNA sequencing demonstrated complete profile of postmortem transcriptome for these psychiatric illnesses.18 Similarly, the studies on transcriptional signature of prenatal human brain (post mortem tissue acquisition) to explore its development and neurodevelopmental disorders, using fluorescence in situ hybridization (FISH); ultrahigh resolution magnetic resonance imaging (MRI) and microarray analysis, illustrated patterned expression of genes related to neuro developmental disorder and human evolution.19

PROTEOMICS

Proteomics is the study of the proteomes. Proteome is the entire set of proteins that is, or can be, expressed by a genome in a cell, tissue or organ at a certain time under defined conditions. Proteomics determine amino acid sequence, modifications, structure and possible pathway in the cell which can aid in the development of new biomarkers in forensic medicine.20 The proteomics based techniques mainly are LC-MS (liquid chromatography-mass spectrometry), SELDI (surface-enhanced laser desorption/ionization), MALDI (matrix assisted laser desorption/ionization), ELISA, MSIA (mass spectrometric immunoassay) etc.21-24

APPLICATION OF PROTEOMICS IN FORENSIC MEDICINE

The detection of the biological fluids

The body fluid identification methods using proteomics approaches driven by the importance for forensic applications have been developed in recent years. Proteins in biological fluid such as blood, saliva, lacrimal fluid, seminal fluid, vaginal fluid, sweat and urine on a surface during scene investigation or on a body part were detected and identified using proteomics strategies. The most important advantage of the proteomics tools was that it did not cause destruction of the DNA. The mass spectroscopy based proteomic approaches can directly detect the origin of specimens and identification of body samples rather than the conventional method of using luminol for blood, specific light sources for saliva and semen followed by their confirmation via immunochemical methods or ELISA.25

PMI

The post mortem degradation of skeletal muscle protein in porcine muscles using protein analysis techniques such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), western blot and casein zymography (an electrophoretic technique) was studied to calculate post mortem interval. It was found that certain muscle proteins like desmin, titin, cardiac troponin-T and sarco-endoplasmic reticulum Ca2+ ATPase (SERCA1) decreased regularly and expectedly. The analysis of the human postmortem skeletal muscle samples also showed similar results to previously conducted porcine study testifying a regular and anticipated protein degradation processes in human muscle.26

Post mortem protein changes to detect new possible markers for PMI estimation, by studying the proteomic profiling of rat and mouse skeletal muscle samples, found two proteins namely eEF1A2 (eukaryotic translation elongation factor1 alpha2) and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) having consistent postmortem degradation in both rats and mice. It indicated that eEF1A2 and GAPDH proteins can be useful markers for PMI estimation.27 The analysis of bone proteomics using LC-MS/MS to estimate PMI, found several new potential markers such as biglycan known for its role in the bone growth and mineralization.28 However, bone proteins markers could only give a relative estimate of PMI.

Diagnosis of disease and cause of death

The mass spectroscopy based proteomic studies have revealed unknown abnormal protein concentrations...
related to tryptophan hydroxylase (TPH2) and serotonin (5-HT) regulation in sudden infant death syndrome (SIDS). A deficiency of 5-HT and its essential biosynthetic enzyme, TPH2, in the medullary 5-HT system that regulated homeostatic responses during sleep had been observed in SIDS infants. A significant reduction has been noted in five of the six isoforms of 14-3-3 signal proteins (14-3-3 proteins bind a multitude of functionally diverse signal proteins) which were known to modulate TPH2 activity in the gigantocellularis of the medullary 5-HT system among SIDS cases compared to control cases. Proteomics therefore, bears a promising prospective to evaluate risk factors and find out pathophysiological basis in SUD in early period of life.39

Proteomic studies can also be helpful in finding the anaphylaxis related cause of death. The evaluation of postmortem blood concentrations of tryptase and β-trypase (markers for mast cell activation) exhibited very high concentration in case of contrast medium anaphylaxis, less in food anaphylaxis and intermediate in insect bites-related anaphylaxis,30-32

METABOLOMICS

Metabolomics is the large scale study of small molecules commonly known as metabolites. Collectively these small molecules (metabolic intermediates, hormones and other signaling molecules) and their interactions within a biological system are known as metabolomes. Metabolomics recently has gained popularity in forensic medicine due to its faster and reproducible methods picturing biological status of the organism.

Metabolomics studies involved two different approaches namely targeted and untargeted. Targeted studies were based on well-defined metabolites whereas untargeted tended to apprehend all metabolites present in the sample.33 The metabolomics-based strategies used for forensic sample analysis methods are Raman spectroscopy (RS), Fourier transform infrared spectroscopy (FTIR), gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), direct injection mass spectrometry (DIMS), nuclear magnetic resonance (NMR) etc.34

APPLICATION OF METABOLOMICS IN FORENSIC MEDICINE

PMI

The recent scientific studies have shown that metabolomics can be an alternative and a new method both for diagnostics and establishing the cause of death. For example, most of the endogenous mammalian microorganisms live in the gastro-intestinal tract which after death caused bloatting and finally led to rupture of corpse. The bacterial community rapidly turned into complex communities which changed the biochemical and metabolic profiles of body specimens. Metabolomic methods based on such profiles can supply convenient markers important about the PMI and cause of death.35-37

The NMR-based metabolic profiling can furnish valuable information about the estimation of PMI and cause of death. The study using proton (1H) NMR spectroscopy to observe the metabolic alterations after death in rat muscles concluded that the metabolic profiles of the tissues differed correspondingly with the manner of death and coordinated well with PMI.38

The mass spectroscopy methods besides NMR were also highly convenient for evaluating postmortem metabolic profile. The GC-MS/MS analysis of blood collected at certain time of postmortem period in asphyxiated rats showed that many endogenous metabolites altered with time since death.39 The studies, using ultra performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF MS) based metabolomics, to analyze metabolite changes in rat liver with respect to the PMI could categories samples for PMI prediction based on the increasing and decreasing concentration of certain metabolites.40

A GC-MS study on metabolomics of postmortem rat blood has observed that twenty six metabolites such as GSH, GABA, glyoxylate, oxalate, hydroxyproline, creatinine, α-ketoglutarate, succinate and 18 amino acids increased with time and were consequently potential biomarkers for determination of PMI.41 The production of formic acid in the blood and urine of corpses had also been analysed using GC-MS and an increase in the concentration of formic acid had been observed.42

The blood pH and changes in concentration of six metabolites-lactic acid, hypoxanthine, uric acid, ammonia, NADH and formic acid were examined post mortem over a 96 hour period in blood taken from animal corpses of rat and pig. The pH and the concentration of these metabolites (which were assayed by using spectrophotometric and assay kits) changed post mortem but the extent and rate of change varied.43 This clearly demonstrated that metabolites have potential for estimating PMI, though comparatively few classes of metabolites have been explored as potential PMI markers.

Intoxication and abuse of drugs

Metabolomics had recently been used to study xenometabolome, the detection of urinary drug metabolites. The MS based metabolomic approach had been explored in xenobiotic metabolic profiling to detect drug metabolites to achieve quick identification of drug. It provided insight in forensic medicine to find out the time of intake of drug and PMI by taking into account all the possible pharmacokinetic of a drug.44 Studies using UPLC/Q-TOF MS based metabolomics for screening and confirmation of various drugs of abuse and their
metabolites in urine in fact demonstrated much better precision than conventional immunoassay methods.45

The metabolomic studies of hair and nails besides urine and blood have also been carried out to detect metabolites of abused drugs or exposed toxic agents keeping in view that the detectability time of metabolites in hair and nails was relatively long, months and years respectively. It carried great significance when the drug and its metabolites have been eliminated (by chemical degradation) from the commonly analyzed body fluids such as urine or blood which makes the metabolomic profiling of hair and nails the most appropriate alternative for interpretation of forensic cases. The study using LC/Q-TOF MS to detect different drugs analytes concluded that nail samples from different post mortem cases provided equivalent results in determining the long-term exposure when the enough amount of hair sample was not available.56

**Diagnosis of disease and cause of death**

The metabolomics can also be utilized for diagnosis of a disease or cause of death based on its ability to disclose a candid picture of metabolic profile. Metabolic profiles have been done for diseases such as Parkinson’s disease, Alzheimer’s disease, intrauterine growth restriction (IUGR), preeclampsia, anaphylaxis.57-51 The cerebrospinal fluid analysis by 1H NMR spectroscopy of autopsy cases of neuropathologically confirmed Alzheimer’s disease showed decrease in citrate levels in comparison to control samples.52 The studies on metabolic profile of anaphylaxis in animal model to look for possible markers using GC-MS showed major metabolic alterations connected with energy metabolism and signal transduction in anaphylaxis.53

**CONCLUSION**

The cutting edge technologies of metabolomics, proteomics, transcriptomics, genomics are bringing new opportunities to promote and reinforce evidence-based evaluation of medico-legal questions in trauma cases, crime scene related issues, the cause, mechanism and the manner of death. The multi-omics analysis when clubbed together into routine forensic methodology has the potential to supplement accurate and precise data to conduct a more efficient forensic investigation. Additional efforts are required to develop the analytical infrastructure necessary to generate, analyze and interpret the multi-omics data effectively to perform precision based omics decision making into routine medico legal applications.

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