CHAPTER 14

Emerging Technologies in Toxicology: Advancements in Risk Assessment

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SUMMARY

Toxicology explores the impact of harmful substances on health and the environment, bridging chemistry, biology, and medicine. This field is crucial for safeguarding public health by identifying and mitigating potential hazards from chemicals and pollutants. Recent advancements in toxicology have significantly enhanced risk assessment methodologies. The integration of high-throughput screening and computational models has improved the prediction of toxic effects, reducing reliance on animal testing. Omics technologies, including genomics and proteomics, provide detailed insights into molecular responses to toxicants. The development of adverse outcome pathways (AOPs) offers a structured framework to understand the progression of toxic effects from molecular initiation to adverse outcomes. Additionally, the incorporation of big data analytics and machine learning enables more accurate and comprehensive risk evaluations, ensuring better protection of human health and the environment. Emerging technologies in toxicology are driving significant advancements in risk assessment. Organs-on-chips technology simulates human organ systems, allowing researchers to study the effects of toxins on specific organs in a controlled and human-relevant manner. CRISPR-Cas9 gene editing enables precise modifications to study genetic variations and their role in toxic responses, enhancing our understanding of genetic susceptibility. Single-cell RNA sequencing reveals cellular heterogeneity and detailed responses to toxic substances at the single-cell level. Therefore, in this chapter we will comprehensively explore how emerging technologies are revolutionizing toxicology and enhancing risk assessment.

INTRODUCTION

The Toxicology is termed as the science of poisons, meaning this branch of science deals with poisons and their impact on life forms. This field deals with the analysis of toxins and their impact on physiology and behavior as well as the assessment and resultant control/treatment of intoxication through qualitative/quantitative methods. Toxicology as a science is thought to have been started by Paracelsus in the 16th century with the death of Orfila in the 19th century; however, the use of poisons is almost as old as civilization. The twentieth century marked significant advancements in our understanding of toxicity, with toxicology expanding into three main specialties: such as its use in environmental, medicinal, and forensic applications because of its extensive impact (Langman & Kapur, 2006).

Contemporary toxicology and analysis are based on highly developed methods of research and assessment of the impact of various substances. The specialized branch of toxicogenomic evaluates toxicity using high throughput methods, computational methods, and genomics (Dai & Shen, 2022). Recent innovations in metabolomics, proteomics, transcriptomics, and computation techniques have made way for flexible risk assessment methodologies (Merrick, 2019). In toxicology, genomic technologies, associated with bioinformatics helped to achieve progress in such areas as toxicogenomic, pharmacogenomics, personalized medicine, genomics, and occupational toxicology, which allowed to work more individually with toxins (Verga et al., 2022; Liu et al., 2019).

It has become evident that the 'omics of the biotechnologies have made toxicity research more efficient as the traditional methods are now comparatively less efficient. Highly efficient genomic, metabolomic, proteomic, toxicogenomic, and lipidomic technologies provide wide capacities to organize and analyze various data sets related to living organisms (Dai & Shen, 2022). Thanks to this expansion in toxicogenomic that has been integrated with digital models even more rapidly (Lin & Chou, 2022). Analytical tools such as machine learning help generate models from multiple sources to support toxicologists in assessing the risks of toxins and any metabolic products associated with chemical compounds and substances like pharmaceuticals and pollutants, expounding experimental toxicology.

OMICS TECHNOLOGIES

Omics technologies have thus transformed how information is collected and analyzed in bioinformatics to describe the components and behavior of particular species

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and their parts. They have also progressed to cover multiple forms of chemical undergoes (e. g., stages of the interactome), disease indicators (e.g. immunome and metabolome), and multiple omics on the epigenetic level, including epi-proteome, epi-transcriptome, and epigenome. Proteomics and metabolomics are among the subsequent omics for which mass spectrometry and sequencing are considered to be key experimental approaches in living organism's analysis (Gysens et al., 2022). Genomic and epigenomic related sequencing technologies are other identified methods while mass spectrometry includes proteomics and metabolomics (Fig 1).

Genomics

Genomic Molecular techniques have the objective of whole-genome sequencing of the genome of interest to describe interspecific relationships at both the soma and germ cells. It has been used to classify many genetic disorders such as those related BRCA1/2 mutations and segmental duplication which constitutes more than 5% of the human genome. They have also been applied to the differentiation of intertypic differences in sequences like the bovine papillomaviruses (Gysens, et al., 2022) and the CoVid-19 virus SARS-CoV-2 variants (Kuchinski, et al., 2022). In the same regard, genomic sequencing also aids in studying the multiple factors of several other complicated diseases including thyroid carcinomas (Iqbal et al., 2022) and endometrial cancers (Hong et al., 2022) and identification of new alleles of polymorphic genes in the human genome.

First-generation technologies include DNA microarray (Thomas et al., 2020), Sanger sequencing (Moniruzzaman et al., 2020); second-generation one is parallel sequencing also known as advanced generation sequencing, NGS (Chen et al., 2021); and third generation is long read sequencing, TGS (Ou et al., 2020). Due to these techniques, the whole exome or NGS genome sequencing has significantly increased the speed and adaptability of gene sequencing, which is mainly categorized into four types: some of the methods of sequencing are as follows cyclic-array sequencing (Shendure et al., 2005; Margulies et al., 2005), micro electrophoretic approaches (Blazej et al., 2006) sequencing by cross-fertilization (Gresham et al. , 2008), and Real time observation of individual molecules (Soni & Meller, 2007).

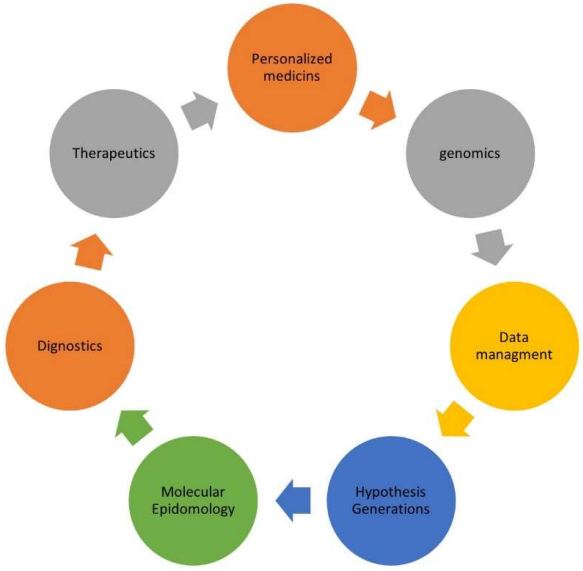

Fig 1. Digital toxicology in handling patient 'OMICS' data and storage to monitor the health and detect toxins response

Some of these methods are practiced in such commercial devices as the Illumina Genome Analyzer (Illumina, USA) (Bentley et al., 2008), the SOLiD platform (Applied Biosystems, USA) (Valouev et al., 2008), and the 454 Genome Sequencers (Roche Life Science, USA) (Shendure et al., 2005). The following products have positively impacted on the omics field. However, the Roche 454 Genome Sequencers and the SOLiD platform were retired in the market chiefly due to low acceptance and Illumina remained on top.

Proteomics

Proteomics examines how data flow through protein signaling (Petricoin et al., 2002) to determine the practical significance of every protein expression in cell, tissue and organismic level, a term known as the proteome (Richard & Horgan, 2011). Since proteins are responsible for most biological functions, it is critical to accurately assess changes in the proteome when cells undergo state transitions, similar to oncogenesis. Transcriptomics is the study of an organism's entire RNA content. Transcription is a process by which the information encoded in DNA is expressed, and it represents an instance in time when the cell is active. Recently, RNA sequencing and microarrays are employed for evaluating transcriptomes. A technique for measuring the expression profile of several genes at once is the microarray. Long sequences of few nucleotides, known as probes, make up microarrays. These sequences can be either long (100–150 bp) or short (20–30 bp). To hybridize with fluorescently labelled transcripts, probes are formed (Mantione et al., 2014). The desired transcript is present in the sample because of this hybridization, and fluorescence intensity is associated with the number of transcripts at each probe position. A sample that is microgram-sized is needed for microarrays. Next-generation RNA sequencing technology with high throughput has replaced microarrays, which require previous knowledge of the organism in order to make probe and more sample. Transcript amount and existence in the RNA extract are disclosed using RNA-Seq (Kukurba & Montgomery, 2015). The next-generation sequencing technique called RNA sequencing can be used for identifying specific active genes at any particular location (Fig 2, Table 1). One of the steps in the sequencing process is the mechanical splitting of cells or tissue to retrieve an RNA transcript. The most extensively researched RNA species are rRNA transcripts, which make up approximately 95 percent of the total proportion of RNA that becomes integrated into ribosomal proteins Yet, only these transcripts such as mRNA represent cellular activity which are of great interest in RNA-Seq. rRNA requires to be eradicated from the pool in order to investigate other RNAs, as it is otherwise generating the majority of sequence reads. The experiment's sensitivity can be raised by enriching the percentage of RNA content and decreasing the non-targeted

transcript. A transcript must be fragmented into smaller transcripts in order to be eligible for sequencing since the mRNA transcripts are larger than the read length of the RNA sequencing technology.

Bioinformatic tools analyze the vast amount of raw data generated by NA sequencing operations to produce meaningful insights. Checking the raw data's quality for base call quality, GC content, short sequence motif delineation (k-mer) as well as duplicate reads to look for impurities, PCR errors, or successive sequencing errors is the first stage in data analysis (Conesa et al., 2016). For sequence quality analysis, a number of software programmers, such as FastQC and FaQCs, are available (Lo & Chain, 2014). Next, transcript sequences are aligned using alignment software to a reference genome. Sequences that are aligned are assessed at the transcript, exon, or gene level. After quantification, data is normalized and statistically analyzed by using EdgeR, Cuffdiff2, Limma/Voom or DEseq2 software to investigate differential gene expression. One method for evaluating the results of a transcriptomics analysis is qPCR. It is possible to quantify the expression of both control and interest genes.

By examining total mRNA, meta transcriptomics also known as profiling of gene expression of microbiomes provides a description of the genes that are active at a given time and in a given environment. A meta transcriptomics investigation of the intestinal microbiota of patients with colorectal cancer revealed the upregulation of many toxin genes that may have carcinogenic potential (Dutilh et al., 2013). Numerous uses for proteome interrogation exist, including the discovery of new ceramide-binding proteins (Bidlingmaier et al., 2016), the examination of Ophiocordyceps sinensis at various stages of culture (Zhang et al., 2020), and the investigation of the proteome landscape of cardiometabolic disorders (Gilly et al., 2020).

Metabolomics

A cell's metabolome is the collection of all biomolecules that make up the cell, with the exception of metals, transcriptome, proteome, and genome. Clish (2015) defines metabolomics as an emerging field that promises to revolutionize precision medicine and is the complete examination of metabolites within a biological material. In the past, monogenic disorders such inborn errors of metabolism and complicated metabolic diseases have been diagnosed using modest amounts of metabolites while modern metabolomic technologies can precisely evaluate hundreds to thousands of metabolites, which are far beyond the reach of conventional clinical chemistry procedures. Metabolomics provide precise characterization of metabolic phenotypes and can facilitate precision medicine on several fronts: identifying novel therapeutic targets, characterizing the metabolic disturbances underlying disease, and finding biomarkers that could be utilized for disease diagnosis or therapeutic activity monitoring. Among the techniques used to investigate the metabolome are liquid chromatography (GC)-MS (Taya et al., 2021; Hayashi et al., 2021), gas chromatography (GC)-MS (Taya et al.,2021; Hayashi et al., 2021), Fourier transform-infrared (FT-IR) spectroscopy (Neto et al., 2022), Raman spectroscopy (Lin et al., 2020), NMR spectroscopy (Eom et al., 2021; Scott et al., 2021), and MS-based techniques like MS (20), MS/MS (Ceglarek et al., 2009). Metabolome profiling has enabled researchers to identify the metabolic process influencing the efficacy of therapy (Geng et al., 2021) as well as potential genes and metabolites (Meng et al., 2021).

Epigenomics

Gene transcription is mostly regulated by changes in the determination of gene activities, without changing the gene sequences are explained by epigenomics (Piunti & Shilatifard, 2016). Histone modification, chromatin reorganizing, DNA methylation, and noncoding RNA-associated procedures are among some of the well-studied epigenetic modifications that are assumed to start and maintain epigenetic modifications (Egger et al., 2004). The identification of DNA/RNA modifications such DNA/RNA methylation and higher-level chromatin structure, which together make up the DNA-DNA interactive (Wang & Chang, 2018). The epigenome varies depending on the kind of cell, and within a single cell, it may affect gene expression in a number of ways, including arrangement of nuclear formation in chromosomes, inhibiting or promoting the transcription factors from the assessment of DNA, and influencing the gene expressions. Gene expression can potentially be significantly impacted through chemical modifications to several DNA bases, as indicated by DNA methylation (Rivera & Ren, 2013). Thus, long-read sequencing methods notably Oxford nanopore sequencing and PacBio have been modified for epigenome interrogation. PacBio SMRT, which tracks the real time polymerase while sequencing DNA, finds epigenomic changes by tracking the

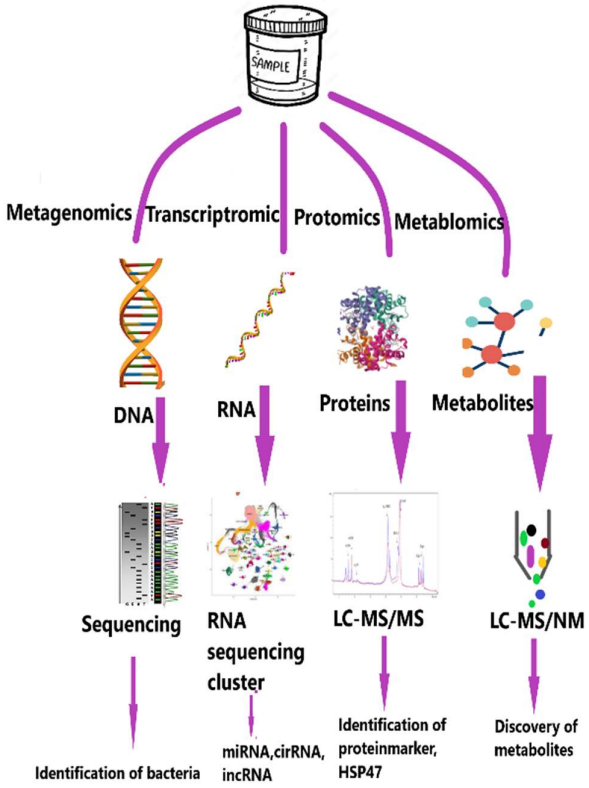

Fig 2. Schematic representation of various omics techniques for toxicant exposure risk assessment

intervals between pulses in a polymerase's reading rate caused by kinetic fluctuation (Li et al., 2018). The implementation of epigenomic sequencing technologies has made it possible to infer epigenomic cell-state dynamics (Farlik et al., 2015), accomplish efficient epigenomic diagnosis of malignancies in brain (Euskirchen et al., 2017), and perform the non-damaging epigenetics molecular phenotyping of human brain (Pan et al., 2020).

Furthermore, Mammals depend on epigenetic regulation for many distinct activities, including development, cell differentiation, and proliferation (Zhu et al., 2013).

HIGH-THROUGHPUT SCREENING

In the fields of pharmaceutics, high-throughput screening (HTS) is gradually occupying precedence of the conventional "trial and error" method for innovative drug development in order to find therapeutic targets and validate biological effects (Thomford et al., 2018; Al-Ali, 2016). In HTS, several biological effectors and modulators are screened and assayed against specific, exclusive targets. As a result, HTS is typically preferred when target knowledge is limited, and structure-based drug design is not feasible. However, HTS can also be utilized in conjunction with other approaches including computational methods and fragment-based drug design (Johnson & Hung, 2019; Parker & Pratt, 2020). Target identification, compound administration, reagent preparation, assay creation as well as screening itself comprises a number of steps that comprise constitute HTS (Noah, 2010). When combined with multi-well cell-based platforms, HTS enables the discovery of small molecule modulators of associated signal transduction and metabolic pathways. Standardized in silico techniques are required to anticipate and restrict interfering compounds being misinterpreted in fluorescence assay technologies, given the significance of HTS platforms in drug discovery and chemical toxicity screening, as well as the possible impact of false signals originating from these two main interference mechanisms (Martis et al., 2011)

COMPUTATIONAL TOXICOLOGY

Conventional assessment of the toxicity of pharmaceuticals, xenobiotics, and environmental contaminants frequently rely on costly, time-consuming, and animal welfare-raising toxicological testing carried out in animal models (Balbus, 2005). It is no longer feasible to evaluate tens of thousands of novel chemicals using animal models due to the rapidly growing number of chemical compounds in industrial, pharmacological as well as agricultural industries (Bassan et al., 2021).

An alternate method that shows promise for predicting the toxicity potentials of compounds is computational toxicology, which utilizes the use of deep learning (DL) and machine learning (ML) (Ciallella & Zhu, 2019). Chemical risk assessments that use ML- and DL-based computer models are impeded by the fact that numerous toxicity models are "black boxes" that are challenging for toxicologists to comprehend. This is true even though the applications of such models in chemical toxicology assessments are alluring. Advancement in deep learning (DL) and machine learning (ML) techniques have made it possible for computational modelling to utilize large amounts of toxicity data to produce more precise chemical toxicity predictions (Xu et al., 2017). It has been demonstrated that certain DL models are comparable to or better than other ML algorithms in terms of prediction accuracy. The urgent requirement to identify the basic mechanisms behind toxicity and explicate the domain studies of toxicological models is being met by the recent developments in interpretable machine learning (IML) in the field of computer science (Jia et al., 2023). Furthermore, these techniques have made it possible for computational modelling to utilize large amounts of toxicity data to produce more precise chemical toxicity predictions. Machine learning (ML) is the process of creating prediction models by employing computational algorithms to learn from input feature data (Jordan & Mitchell, 2015; Zhou, 2022). The process of creating a predictive model usually entails gathering and evaluating data, creating the model, and validating the model. Various data formats offer distinct characteristics and varying degrees of user interpretability. It has been demonstrated that certain DL models are comparable to or better than other ML algorithms in terms of prediction accuracy.

ML models are widely employed in both daily activities (e.g., advertising, hiring and music recommendations) as well as health-associated domains (i.e., drug development, health maintenance and risk assessment) (Koenigstein et al., 2011; Linden et al., 2003; Dattner et al., 2019). However, utilizing black box models without knowing how they operate can make results unreliable. Black box models have a negative impact on safety, racial bias, and human health in disciplines associated with health (Lo Piano, 2020). IML has the capability of conducting rigorous model validations to prevent making incorrect judgements based on skewed training data and to elucidate the underlying process of decision-making. (Guidotti et al., 2018) In addition to a model's predictions, the information regarding chemical toxicants incorporated into the training data would enable toxicologists to assess the trained model more effectively as well as make judgements regarding novel compounds—that is, identify which pharmaceuticals and environmental chemicals pose the greatest risk to human health (Murdoch et al., 2019).

Model training and posthoc interpretability are two different ways that ML models can be made interpretable (Murdoch et al., 2019; Du et al., 2019). The two types of interpretabilities can be attained to accomplish intrinsic interpretability, self-explanatory models that integrate interpretability directly into the model structures (e.g., toxicological knowledge base frameworks) are constructed.

IN VITRO AND ORGAN-ON-A-CHIP TECHNOLOGIES

An interdisciplinary technology known as organ-on-a-chip (OOC) has developed into a modern in vitro model for pharmacokinetic and pharmacodynamic (PK-PD) research of a suggested drug candidate during the pre-clinical stages of drug development (Joseph et al., 2022). The OOC serves as a platform that simulates physiological processes which happen

Table 1. Summarized advantages and disadvantages of omics technologies

Omics technologies	Advantages	Disadvantages	Citations
Genomics	Analysis of the nucleotide sequences, genome structure and its composition. SNPs are important tools to find out the risk of acquiring disease. For example. o Affymetrix SNP GeneChip and IIIumina Golden Gate Bead Chips assays	It is difficult to predict the final biological effect of variation/s in DNA by only genome analysis because of posttranscriptional and post-translational changes, epigenetics For example, o NGS, PCR, RFLP-PCR techniques	Vucic et al., 2014; Jiang et al., 2016
Transcriptomics	predict the changes on protein levels and activities Identification of the major pathways involved in drug response and toxicity	Inadequate data due to post-translational modifications. Changes in the transcriptome are not able to show a variation in the pattern of "end products".	Jiang et al., 2016; Dong et al., 2016
Proteomics and Metabolomics	Provides the quantitative comparative analysis using a single gel. Eliminate post-electrophoretic processing steps such as fixing and destaining	Proteins without lysine cannot be labeled. Cost is increased due to requiring special equipment	Fiehn, 2002
Proteomics	High throughput (detection of hundreds of individuals species within a single sample)	lack of information on protein since the Peptides might not have all come from a single protein species.	Messner et al., 2023

to place in the human body. OOC is considerably more comprehensive than traditional two-dimensional (2D) culture systems due to its accurate flow control systems and quick sample processing. Additional insights into the time- and dose-dependent effects that an innovative pharmaceutical molecule will have on the body during pre-clinical testing are likely to be acquired from the integration of several organs, as demonstrated by the multi-organs-on-a-chip. The rapid development of organ-on-a-chip technology triggered a paradigm shift in the traditional in vitro assays for toxicity evaluations and absorption, distribution, metabolism, and excretion (ADME) research. The primary focus of any drug delivery mechanism is currently a requirement for an accurate prediction model for detecting toxicity and diminishing potential risk factors. The use of organ-on-a-chips for ADME and toxicity assessment is being applauded by researchers worldwide (Parker & Pratt, 2020; Dahlin et al., 2015).

Furthermore, OOCs are devices that are used in the fabrication process by employing the microfluidic technology for generating human organ-like cell cultures. In order to replicate the functionality of an organ, microfluidic devices can help with the reconstruction of multi-scale architecture, tissue-tissue interaction by using mechanical cues, and chemically and physiologically appropriate microenvironments (Aziz et al., 2017).

IMAGING TECHNOLOGIES

A number of advanced novel methods of imaging technology have been designed to allow the acquisition of high-resolution or three-dimensional (3D) digital images. Most of these micro imaging technologies can provide detailed visualization even at subcellular stages particularly in the realm of biological and medical domains. A set of technologies known as "emerging technologies" are used in the regulatory field to add or to replace the typical toxicological evaluations carried out in support of an Investigational New Drug Application (IND). The basic program comprises a range of toxicological research of various sizes, safety pharmacological research, genetic toxicological as well as research focused on reproductive toxicity (Leighton, 2005). Some of the modern imaging methods are:

Confocal microscopy

Confocal microscopy is an optical imaging technique used to increase optical resolution, primarily generate a focused light point and enable the internal tissues at considerable depth with higher resolution (Zhang & Monteiro-Riviere, 2013). Confocal microscopy quantified extensive inflammatory response and collagen development in the lung, visceral and parietal pleura as well as pleural adhesions (Bernstein et al., 2021). In the experimental study, we were able to identify and evaluate the effects of exposing Danio rerio's diet to various kinds of microplastics (De Sales-Ribeiro et al., 2020). MPs are unlikely to get accumulated in the digestive tract, suggesting that their accumulation rate is minimal (Grigorakis et al., 2017; Jovanović et al., 2018; Lu et al., 2016). However, larger particles exhibit prolonged retention within GIT. Confocal microscopy was used to evaluate the existence and absorption of fluorescent microplastics (MPs) by various tissues. Fluorescent images were captured using a confocal microscope (Zeiss Confocal LSM800, Germany). Complete panoramic images of the entire intestine of *D. rerio* were observed. To confirm the absorption of microplastic particles into tissues, a sequence of two- dimensional images covering specific depth ranges (Z-stacks) was conducted to generate three-dimensional image (De Sales-Ribeiro et al., 2020).

MRI

Magnetic resonance imaging (MRI) is a medical as well as radiological method used to produce visual representation of anatomical and physiological functions of the body. It plays a

vital role in prognostics, diagnostics and in basic scientific discoveries (Beuf et al., 2006) of many animals like polychaetas (Dinley et al., 2010) and echinoderms (Ziegler et al., 2010; Ziegler et al., 2008). Moreover, it has the ability to produce clear images of soft tissues by using a powerful magnetic field to align the rotation of hydrogen nuclei of study object in various planes. By encoding the frequency of rotation, external field strength and spin phase in three-dimensional space, this technique splits the study object into voxels to generate comprehensive images. For example, the administration of MRI contrast agent to the white knee tarantula specie orally amplified the signals that could be detected from digestive tract. Furthermore, it is a non-intrusive technology that typically maintains the animal's integrity e.g. examination of tissue regeneration and volumetric alterations in gastrointestinal tract throughout the process of digestion. Nevertheless, MRI demands complete immobilization of animal throughout the entire scanning process directing the respiratory or heartbeat stimulation in order to minimize most artifacts. Both in the field of oncology and specifically in neuro-oncology, the assessment of therapeutic efficacy or observation of tumor therapy holds significant importance. For every decennial, alterations in the range of contrast enhancement on MRI have conventionally served as an indicator of response therapy or the recurrence of brain tumors in patients (Wen et al., 2010).

PET

Positron emission tomography (PET) scans early signs of tumor, brain disorder and also involve in the administration of harmless radioactive tracer helping in the detection of affected cells. PET is one of the most promising methods for visualization of specific molecular procedures within living organism. In this procedure, biologically active compounds are marked with short-lived positron-emitting isotopes at concentration measured in micromoles or nanomoles. Historically, the primary and widely used PET tracer for oncologic imaging has been 18F-2-fluoro-2-deoxy- D-glucose (FDG). FDG tends to accumulate in majority of tumors because of increased energy requirements leading to increased glucose metabolism but there are some limitations in the use of FDG. Several studies have reported the correlation between FDG uptake, glioma tumor grade and prognosis (Herholz et al., 2012). The most extensive knowledge regarding this category of PET tracers used for imaging of brain tumor has been acquired with MET. This particular tracer is an essential amino acid labelled with the positron-emitting carbon- 11 isotopes having a half-life of 20 minutes (Galldiks et al., 2015b; Herholz et al., 2012). This comparatively short half-life restricted the application of MET to PET centers that have on site cyclotron unit. For instance, FET represents an amino acid tracer labeled with 18F with half-life of 110 minutes and developed in the late 1990s, providing logistical benefits for clinical applications over MET (Langen et al., 2006; Wester et al., 1999). In recent years, the use of FET has increased significantly particularly in Western Europe. The clinical outcomes for PET-using MET and FET in brain cancer patients seem identical (Grosu et al., 2011; Langen et al., 2003; Weber et al., 2000).

BIOMARKERS AND MOLECULAR SIGNATURES

Identification and validation of biomarkers for toxicant exposure and toxicity

Biomarkers are considered as environmental toxicant which is used to assess and evaluate the interindividual exposure and effects of disease risk. Proteins, metabolites and DNA conjugates play an important role in understanding the mechanism of activation and detoxification of reactive intermediates of toxicants (Lagoa et al., 2022). Moreover, in environmental toxicology, it is a measurable parameter of harmful effects in living cell that can be applied as an indicator of chemical exposure (Van der Oost et al., 2003). It has been suggested that molecular biomarkers including gene transcripts can detect responses to damaging effects even at organism level (Calzolai et al., 2007; Piña et al., 2007). Although there are certain transcripts that are thought to be potential indicators of general toxicity and chemical stress such as responses related to toxicant metabolism, oxidative stress and overall cytoprotecting while others show specificity to particular toxicant (Bultelle et al., 2024; Sulmon et al., 2015). This viewpoint summarizes the information currently available on biomarkers of exposure in ENDS (electronic nicotine delivery system) users in order to support the comprehensive evaluation of health effects. Since there are a number of renowned sources of chemicals released from ENDS devices it is still difficult to identify novel biomarkers of exposure that are unique to ENDS users. The levels of biomarkers of numerous tobacco-related toxicants observed in biological samples of ENDS addicts showed no significant differences as compared to non- addicts (Drope et al., 2017). With the exception of nicotine metabolites and a few biomarkers of exposure to tobacco-specific nitrosamines and volatile organic compounds. Numerous studies have been revealed that the long-term exclusive electronic nicotine delivery system (ENDS) users exhibit significantly lower levels of toxicant biomarkers than cigarette smokers while exposed to nicotine. Research has also demonstrated that individual who use both ENDS and combustible cigarettes simultaneously commonly referred as "dual users" do not experience a reduction in overall exposure to harmful toxicants when compared to individual exclusively using combustible cigarettes (Goniewicz, 2023).

Molecular signatures to assess the effects of toxicants

Applications of predictive biology are increasingly incorporated the molecular signature. Nevertheless, there is insufficient research about the overall predictive effectiveness of transcriptomics and epigenomics regarding perinatal outcome (Clark et al., 2021). One of the genomic approaches, toxicogenomic is used to predict the detrimental impacts of exposure to external stimuli are getting popular with advancement in estimating and availability of data set. Toxicogenomic databases have been developed and through extensive research on rats and human cell it provides an opportunity of recognizing the genetic basis of damaging effects caused by exposure to toxic compounds (Maggioli et al., 2006). Metabolomics is the systematic analysis of low molecular metabolites in biological systems such as tissues,

urine or body fluids. Changes in metabolome are caused by biochemical reactions triggered by catalytic protein in response to environmental factor (Wishart et al., 2012). Occupational diseases are more likely to occur in individuals who are exposed to chemicals in the workplace (Boschetto et al., 2006). Occurrence of occupational diseases tend to rise with work related exposures to harmful agents or factors. These exposures typically involve a complex mixture of various stressor making it difficult to identify the risk factors and mechanisms underlying the relationships between exposure and disease. Omics approaches enable the global visualization of internal molecular changes. These indicators may be used to determine the bio effects of a toxicant exposure (Dehghani et al., 2022).

Nitrosamine, a particular group of organic compound commonly found in aquatic environment especially in large water bodies (Chen et al., 2018; Dai & Mitch, 2013) The metabolic disturbances in human esophageal epithelial Het-1A cells caused by a combination of nine basic nitrosamines in clear water at naturally sustainable environment. A person's internal susceptibility and genotoxic level were examined using metabiological approach. In general, as the dose of nitrosamines increased, the disturbed metabolic profile got more complex even at the environmental level (He et al., 2023). Notably, at this environment related level nitrosamines significantly changed the two inflammation-related avenues: named as, the metabolism of cysteine (Cys) and methionine (MET), as well as the metabolism of nicotinate and nicotinamide. Moreover, simultaneous identification of molecular markers and targeted metabolomics in mouse cells revealed the up regulation of Cys (cystien) and Met (methionine) that ultimately gives methyl donors for histone methylation in terms of pro-inflammatory response. Subsequently, the NF-kβ signaling pathway was subsequently activated due to the restriction of NF-kβ p65's deacetylation by the downregulation of NAD+/NADH ratio. When taken together, the metabolomics molecular signatures served as significant suggestive indicator for inflammation induced by nitrosamines (Zhao et al., 2022).

CONCLUSION

Several of the most efficient emerging technologies in toxicology include omics technologies, organ on a chip models, high-throughput screening methods, and computational toxicology as well as advanced imaging techniques for enhanced the speed and accuracy of toxicity evaluations, faster screening of large datasets and improved understanding of complex interactions. Genomics, Proteomics, transcriptomics and metabolomics play a crucial role in understanding the molecular mechanisms of toxicity at a comprehensive level while modeling and simulation techniques help to predict toxicity for more efficient prioritization of chemicals for further testing. Microfluidic devices replicate organ functions allowing more accurate toxicity testing on human cells as well as biosensors detect and quantify toxic substances with sensitivity, offering real time monitoring capabilities. Embracing the latest technology in toxicology is crucial for advancing safety assessments, improving predictive capability as well as reducing the reliance on traditional animal testing. These technologies lead to more efficient drug development processes and contributing to the perfection of safer products. Additionally, these technologies support the ethical considerations associated with minimizing animal use in research while providing an efficient practice in toxicology.

REFERENCES

Al-Ali H, 2016. The evolution of drug discovery: From phenotypes to targets, and back. MedChemComm 7:788-98. https://doi.org/10.1039/C6MD00129G

Aziz AUR, C Geng, M Fu et al., 2017. The role of microfluidics for organ on chip simulations. Bioengineering 4:39. https://doi.org/10.3390/bioengineering4020039

Balbus JM, 2005. Ushering in the new toxicology: Toxicogenomics and the public interest. Environmental Health Perspectives 113:818-22. https://doi.org/10.1289/ehp.7732

Bassan A, VM Alves, A Amberg et al., 2021. In silico approaches in organ toxicity hazard assessment: Current status and future needs in predicting liver toxicity. Computational Toxicology 20:100187. https://doi.org/10.1016/j.comtox.2021.100187

Bentley DR, S Balasubramanian, HP Swerdlow et al., 2008. Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456:53-9.

Bernstein DM, B Toth, RA Rogers et al., 2021. Final results from a 90-day quantitative inhalation toxicology study evaluating the dose-response and fate in the lung and pleura of chrysotile-containing brake dust compared to TiO2, chrysotile, crocidolite or amosite asbestos: Histopathological examination, confocal microscopy and collagen quantification of the lung and pleural cavity. Toxicology and Applied Pharmacology 424:115598. https://doi.org/10.1016/j.taap.2021.115598

Beuf O, F Jaillon & H Saint-Jalmes, 2006. Small-animal MRI: Signal-to-noise ratio comparison at 7 and 1.5 T with multiple-animal acquisition strategies. Magnetic Resonance Materials in Physics, Biology and Medicine 19:202-8. https://doi.org/10.1007/s10334-006-0048-9

Bidlingmaier S, K Ha, NK Lee et al., 2016. Proteome-wide identification of novel ceramide-binding proteins by yeast surface cDNA display and deep sequencing. Molecular and Cellular Proteomics 15:1232-45. https://doi.org/10.1074/mcp.M115.055954

Blazej RG, P Kumaresan & RA Mathies, 2006. Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing. Proceedings of the National Academy of Sciences 103:7240-5. https://doi.org/10.1073/pnas.0602476103

Boschetto P, S Quintavalle, D Miotto et al., 2006. Chronic obstructive pulmonary disease (COPD) and occupational exposures. Journal of Occupational Medicine and Toxicology 1:1-6. https://doi.org/10.1186/1745-6673-1-11

Bultelle F, A Le Saux, E David et al., 2024. Cadmium highlights common and specific responses of two freshwater sentinel species, *Dreissena polymorpha* and *Dreissena rostriformis* bugensis. Proteomes 12:10. https://doi.org/10.3390/proteomes12020010

Calzolai L, W Ansorge, E Calabrese et al., 2007. Transcriptomics and proteomics. Applications to ecotoxicology. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 2:245-9. https://doi.org/10.1016/j.cbd.2007.04.007

Ceglarek U, A Leichtle, M Bruegel et al., 2009. Challenges and developments in tandem mass spectrometry based clinical metabolomics. Molecular and Cellular Endocrinology 301:266-71. https://doi.org/10.1016/j.mce.2008.10.013

Chen X, G Huang, C An et al., 2018. Emerging N-nitrosamines and N-nitramines from amine-based post-combustion CO2 capture-a review. Chemical Engineering Journal 335:921-35. https://doi.org/10.1016/j.cej.2017.11.032

Chen X, Y Dong, Y Huang et al., 2021. Whole-genome resequencing using next-generation and Nanopore sequencing for molecular characterization of T-DNA integration in transgenic poplar 741. BMC Genomics 22:1-3. https://doi.org/10.1186/s12864-021-07625-y

Ciallella HL & H Zhu, 2019. Advancing computational toxicology in the big data era by artificial intelligence: data-driven and mechanism-driven modeling for chemical toxicity. Chemical Research in Toxicology 32:536-47. https://doi.org/10.1021/acs.chemrestox.8b00393

Clark J, V Avula, C Ring et al., 2021. Comparing the predictivity of human placental gene, microRNA, and CpG methylation signatures in relation to perinatal outcomes. Toxicological Sciences 183:269-84. https://doi.org/10.1093/toxsci/kfab089

Clish CB, 2015. Metabolomics: An emerging but powerful tool for precision medicine. Molecular Case Studies 1:000588. https://doi.org/10.1101/mcs.a000588

Conesa A, P Madrigal, S Tarazona et al., 2016. A survey of best practices for RNA-seq data analysis. Genome Biology 17:1-9. https://doi.org/10.1186/s13059-016-0881-8

Dahlin JL, JWM Nissink, S Francis et al., 2015. Post-HTS case report and structural alert: Promiscuous 4-aroyl-1, 5-disubstituted-3-hydroxy-2H-pyrrol-2-one actives verified by ALARM NMR. Bioorganic and Medicinal Chemistry Letters 25:4740-52. https://doi.org/10.1016/j.bmcl.2015.08.020

Dai N & WA Mitch, 2013. Influence of amine structural characteristics on N-nitrosamine formation potential relevant to postcombustion CO2 capture systems. Environmental Science and Technology 47:13175-83. https://doi.org/10.1021/es4035396

Dai X & L Shen, 2022. Advances and trends in omics technology development. Frontiers in Medicine 9:911861. https://doi.org/10.3389/fmed.2022.911861

Dattner B, T Chamorro-Premuzic, R Buchband et al., 2019. The legal and ethical implications of using AI in hiring. Harvard Business Review 25:1-7.

De Sales-Ribeiro C, Y Brito-Casillas, A Fernandez et al., 2020. An end to the controversy over the microscopic detection and effects of pristine microplastics in fish organs. Scientific Reports 10:12434. https://doi.org/10.1038/s41598-020-69062-3

Dehghani F, S Yousefinejad, DI Walker et al., 2022. Metabolomics for exposure assessment and toxicity effects of occupational pollutants: current status and future perspectives. Metabolomics 18:73. https://doi.org/10.1007/s11306-022-01930-7

Dinley J, L Hawkins, G Paterson et al., 2010. Micro-computed X-ray tomography: A new non-destructive method of assessing sectional, fly-through and 3D imaging of a soft-bodied marine worm. Journal of Microscopy 238:123-33. https://doi.org/10.1111/j.1365-2818.2009.03335.x

Dong S, QL Chen, YN Song et al., 2016. Mechanisms of CCl4-induced liver fibrosis with combined transcriptomic and proteomic analysis. The Journal of Toxicological Sciences 41:561-72. https://doi.org/10.2131/jts.41.561

Drope J, Z Cahn, R Kennedy et al., 2017. Key issues surrounding the health impacts of electronic nicotine delivery systems (ENDS) and other sources of nicotine. CA: A Cancer Journal for Clinicians 67:449-71. https://doi.org/10.3322/caac.21413

Du M, N Liu & X Hu, 2019. Techniques for interpretable machine learning. Communications of the ACM 63:68-77. https://doi.org/10.1145/3359786

Dutilh BE, L Backus, SA van Hijum et al., 2013. Screening metatranscriptomes for toxin genes as functional drivers of human colorectal cancer. Best Practice and Research Clinical Gastroenterology 27:85-99. https://doi.org/10.1016/j.bpg.2013.03.008

Egger G, G Liang, A Aparicio et al., 2004. Epigenetics in human disease and prospects for epigenetic therapy. Nature 429:457-63. https://doi.org/10.1038/nature02625

Eom JS, ET Kim, HS Kim et al., 2021. Metabolomics comparison of rumen fluid and milk in dairy cattle using proton nuclear magnetic resonance spectroscopy. Animal Bioscience 34:213. https://doi.org/10.5713/ajas.20.0197

Euskirchen P, F Bielle, K Labreche et al., 2017. Same-day genomic and epigenomic diagnosis of brain tumors using real-time nanopore sequencing. Acta Neuropathologica 134:691-703. https://doi.org/10.1007/s00401-017-1743-5

Farlik M, NC Sheffield, A Nuzzo et al., 2015. Single-cell DNA methylome sequencing and bioinformatic inference of epigenomic cell-state dynamics. Cell Reports 10:1386-97. https://doi.org/10.1016/j.celrep.2015.02.001

Fiehn O, 2002. Metabolomics-the link between genotypes and phenotypes. Plant Molecular Biology 48:155-71. https://doi.org/10.1007/978-94-010-0448-0_11

Galldiks N, KJ Langen & WB Pope, 2015. From the clinician's point of view. What is the status quo of positron emission tomography in patients with brain tumors? Neuro-oncology 17:1434-44. https://doi.org/10.1093/neuonc/nov118

Geng QS, RJ Liu, ZB Shen et al., 2021. Transcriptome sequencing and metabolome analysis reveal the mechanism of Shuanghua Baihe Tablet in the treatment of oral mucositis. Chinese Journel of National Medicines 19:930-43. https://doi.org/10.1016/S1875-5364(22)60150-X

Gilly A, YC Park, G Png et al., 2020. Whole-genome sequencing analysis of the cardiometabolic proteome. Nature Communications 11:6336. https://doi.org/10.1038/s41467-020-20079-2

Goniewicz ML, 2023. Biomarkers of electronic nicotine delivery systems (ENDS) use. Addiction Neuroscience 6:100077. https://doi.org/10.1016/j.addicn.2023.100077

Gresham D, MJ Dunham & D Botstein, 2008. Comparing whole genomes using DNA microarrays. Nature Reviews Genetics 9:291-302. https://doi.org/10.1038/nrg2335

Grigorakis S, SA Mason & KG Drouillard, 2017. Determination of the gut retention of plastic microbeads and microfibers in goldfish (*Carassius auratus*). Chemosphere 169:233-8. https://doi.org/10.1016/j.chemosphere.2016.11.055

Grosu AL, ST Astner, E Riedel et al., 2011. An interindividual comparison of O-(2-[18F] Fluoroethyl)-L-Tyrosine (FET)-and L-[Methyl-11C] methionine (MET)-PET in patients with brain gliomas and metastases. International Journal of Radiation Oncology Biology Physics 81:1049-58. https://doi.org/10.1016/j.ijrobp.2010.07.002

Guidotti R, A Monreale, S Ruggieri et al., 2018. A survey of methods for explaining black box models. ACM Computing Surveys 51:1-42. https://doi.org/10.1145/3236009

Gysens L, B Vanmechelen, M Haspeslagh et al., 2022. New approach for genomic characterization of equine sarcoid-derived BPV-1/-2 using nanopore-based sequencing. Virology Journal 19:8. https://doi.org/10.1186/s12985-021-01735-5

Hayashi Y, T Komatsu, K Iwashita et al., 2021. 1H-NMR metabolomics-based classification of Japanese sake and comparative metabolome analysis by gas chromatography-mass spectrometry. Journal of Bioscience and Bioengineering 131:557-64. https://doi.org/10.1016/j.jbiosc.2020.12.008

He L, X Zhou, J Liu et al., 2023. RAE1 promotes nitrosamine-induced malignant transformation of human esophageal epithelial cells through PPARα-mediated lipid metabolism. Ecotoxicology and Environmental Safety 265:115513. https://doi.org/10.1016/j.ecoenv.2023.115513

Healy K, 2007. Nanopore-based single-molecule DNA analysis 2:459-481. https://doi.org/10.2217/17435889.2.4.459

Herholz K, KJ Langen, C Schiepers et al., 2012. Brain tumors. In Seminars in nuclear medicine. Nanomedicine 6:356-70. https://doi.org/10.1053/j.semnuclmed.2012.06.001

Hong JH, HW Cho, YT Ouh et al., 2022. Genomic landscape of advanced endometrial cancer analyzed by targeted next-generation sequencing and the cancer genome atlas (TCGA) dataset. Journal of Gynecologic Oncology 33:29. https://doi.org/10.3802/jgo.2022.33.e29

Iqbal MA, M Li, J Lin et al., 2022. Preliminary study on the sequencing of whole genomic methylation and transcriptome-related genes in thyroid carcinoma. Cancers 14:1163. https://doi.org/10.3390/cancers14051163

Jia X, T Wang & H Zhu, 2023. Advancing Computational Toxicology by Interpretable Machine Learning. Environmental Science and Technology 57:17690-706. https://doi.org/10.1021/acs.est.3c00653

Jiang Z, T Lao, W Qiu et al., 2016. A chronic obstructive pulmonary disease susceptibility gene, FAM13A, regulates protein stability of β-catenin. American Journal of Respiratory and Critical Care Medicine 194:185-97.

Johnson EO & DT Hung, 2019. A point of inflection and reflection on systems chemical biology. ACS Chemical Biology 14:2497-511. https://doi.org/10.1021/acschembio.9b00714

Jordan MI & TM Mitchell, 2015. Machine learning: Trends, perspectives, and prospects. Science 349:255-60. https://doi.org/10.1126/science.aaa8415

Joseph X, V Akhil, A Arathi et al., 2022. Comprehensive development in organ-on-a-chip technology. Journal of Pharmaceutical Sciences 111:18-31. https://doi.org/10.1016/j.xphs.2021.07.014

Jovanovic B, K Gokdag, O Guven et al., 2018. Virgin microplastics are not causing imminent harm to fish after dietary exposure. Marine Pollution Bulletin 130:123-31. https://doi.org/10.1016/j.marpolbul.2018.03.016

Koenigstein N, G Dror & Y Koren, 2011. Yahoo! music recommendations: modeling music ratings with temporal dynamics and item taxonomy. In Proceedings of the fifth ACM conference on Recommender systems (RecSys '11). Association for Computing Machinery, New York, NY, USA, pp: 165–72. https://doi.org/10.1145/2043932.2043964

Kuchinski KS, J Nguyen, TD Lee et al., 2022. Mutations in emerging variant of concern lineages disrupt genomic sequencing of SARS-CoV-2 clinical specimens. International Journal of Infectious Diseases 114:51-4. https://doi.org/10.1016/j.ijid.2021.10.050

Kukurba KR & SB Montgomery, 2015. RNA sequencing and analysis. Cold Spring Harbor Protocols 2015:084970. https://doi.org/10.1101/pdb.top084970

Lagoa R, D Marques-da-Silva, M Diniz et al., 2022. Molecular mechanisms linking environmental toxicants to cancer development: Significance for protective interventions with polyphenols. Seminars in Cancer Biology 80:118-144. https://doi.org/10.1016/j.semcancer.2020.02.002

Langen KJ, K Hamacher, M Weckesser et al., 2006. O-(2-[18F] fluoroethyl)-L-tyrosine: Uptake mechanisms and clinical applications. Nuclear Medicine and Biology 33:287-94. https://doi.org/10.1016/j.nucmedbio.2006.01.002

Langen KJ, M Jarosch, H Mühlensiepen et al., 2003. Comparison of fluorotyrosines and methionine uptake in F98 rat gliomas. Nuclear Medicine and Biology 30:501-8. https://doi.org/10.1016/S0969-8051(03)00023-4

Langman LJ & BM Kapur, 2006. Toxicology: Then and now. Clinical Biochemistry 39:498-510. https://doi.org/10.1016/j.clinbiochem.2006.03.004
Leighton JK, 2005. Application of emerging technologies in toxicology and safety assessment: Regulatory perspectives. International Journal of Toxicology 24:153-5. https://doi.org/10.1080/10915810590948352
Li T, X Zhang, F Luo et al, 2018. MultiMotifMaker: A multi-thread tool for identifying DNA methylation motifs from Pacbio reads. IEEE/ACM Transactions on Computational Biology and Bioinformatics 17:220-5. https://doi.org/10.1109/TCBB.2018.2861399
Lin D, Y Wang, T Wang et al., 2020. Metabolite profiling of human blood by surface-enhanced raman spectroscopy for surgery assessment and tumor screening in breast cancer. Analytical and Bioanalytical Chemistry 412:1611-8. https://doi.org/10.1007/s00216-020-02391-4
Lin Z & WC Chou, 2022. Machine learning and artificial intelligence in toxicological sciences. Toxicological Sciences 189:7-19. https://doi.org/10.1093/toxsci/kfac075
Linden G, B Smith & J York, 2003. Amazon.com recommendations: Item-to-item collaborative filtering. IEEE Internet Computing 7:76-80. https://doi.org/10.1109/MIC.2003.1167344
Liu Z, R Huang, R Roberts et al., 2019. Toxicogenomics: a 2020 vision. Trends in Pharmacological Sciences 40:92-103. https://doi.org/10.1016/j.tips.2018.12.001
Lo CC & PS Chain, 2014. Rapid evaluation and quality control of next generation sequencing data with FaQCs. BMC Bioinformatics 15:1-8. https://doi.org/10.1186/s12859-014-0366-2
Lo Piano S, 2020. Ethical principles in machine learning and artificial intelligence: cases from the field and possible ways forward. Humanities and Social Sciences Communications 7:1-7. https://doi.org/10.1057/s41599-020-0501-9
Lu Y, Y Zhang, Y Deng et al. 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environmental Science And Technology 50:4054-60. https://doi.org/10.1021/acs.est.6b00183
Maggioli J, A Hoover & L Weng, 2006. Toxicogenomic analysis methods for predictive toxicology. Journal of Pharmacological and Toxicological Methods 53:31-7. https://doi.org/10.1016/j.vascn.2005.05.006
Mantione KJ, RM Kream & H Kuzelova, 2014. Comparing bioinformatic gene expression profiling methods: Microarray and RNA-Seq. Medical Science Monitor Basic Research 20:138. https://doi.org/10.12659/MSMBR.892101
Margulies M, M Egholm, WE Altman et al., 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376-80.
Martis EA, R Radhakrishnan & RR Badve, 2011. High-throughput screening: the hits and leads of drug discovery-an overview. Journal of Applied Pharmaceutical Science 1:2-10.
Meng X, J Xu, M Zhang et al., 2021. Third-generation sequencing and metabolome analysis reveal candidate genes and metabolites with altered levels in albino jackfruit seedlings. BMC Genomics 22:1-4. https://doi.org/10.1186/s12864-021-07873-y
Merrick BA, 2019. Next-generation sequencing data for use in risk assessment. Current Opinion in Toxicology 18:18-26. https://doi.org/10.1016/j.cotox.2019.02.010
Messner CB, V Demichev, Z Wang et al., 2023. Mass spectrometry-based high-throughput proteomics and its role in biomedical studies and systems biology. Proteomics 23:2200013. https://doi.org/10.1002/pmic.202200013
Moniruzzaman M, MU Hossain, V Islam et al., 2020. Coding-complete genome sequence of SARS-CoV-2 isolate from Bangladesh by sanger sequencing. Microbiology Resource Announcements 9:10-128. https://doi.org/10.1128/MRA.00626-20
Murdoch WJ, C Singh, K Kumbier et al., 2019. Definitions, methods, and applications in interpretable machine learning. Proceedings of the National Academy of Sciences 116:22071-80. https://doi.org/10.1073/pnas.1900654116
Neto V, S Esteves-Ferreira, I Inacio et al., 2022. Metabolic profile characterization of different thyroid nodules using FTIR spectroscopy: A review. Metabolites 12:53. https://doi.org/10.3390/metabo12010053
Noah JW, 2010. New developments and emerging trends in high-throughput screening methods for lead compound identification. International Journal of High Throughput Screening 141-9. https://doi.org/10.2147/IJHTS.S8683
Ou YJ, QQ Ren, ST Fang et al., 2020. Complete genome insights into Lactococcus petauri CF11 isolated from a healthy human gut using second- and third-generation sequencing. Frontiers in Genetics 11:119. https://doi.org/10.3389/fgene.2020.00119
Pan L, L McClain, P Shaw et al., 2020. Non-invasive epigenomic molecular phenotyping of the human brain via liquid biopsy of cerebrospinal fluid and next generation sequencing. European Journal of Neuroscience 52:4536-45. https://doi.org/10.1111/ejn.14997
Parker CG & MR Pratt, 2020. Click chemistry in proteomic investigations. Cell 180:605-32. https://doi.org/10.1016/j.cell.2020.01.025
Petricoin EF, KC Zoon, EC Kohn et al., 2002. Clinical proteomics: Translating benchside promise into bedside reality. Nature Reviews Drug Discovery 1:683-95. https://doi.org/10.1038/nrd891
Pina-Cabral L, L Santos, B Mesquita et al., 2007. Detection of survivin mRNA in urine of patients with superficial urothelial cell carcinomas. Clinical and Translational Oncology 9:731-6. https://doi.org/10.1007/s12094-007-0130-4
Piunti A & A Shilatifard, 2016. Epigenetic balance of gene expression by Polycomb and COMPASS families. Science 352:9780. https://doi.org/10.1126/science.aad9780
Richard P & LCK Horgan, 2011. 'Omic' technologies: genomics, transcriptomics, proteomics and metabolomics. Obstetrics Gynaecology 13:189-95. https://doi.org/10.1576/toag.13.3.189.27672
Rivera CM & B Ren, 2013. Mapping human epigenomes. Cell 155:39-55. https://doi.org/10.1016/j.cell.2013.09.011
Scott HD, M Buchan & C Chadwick et al., 2021. Metabolic dysfunction in pregnancy: Fingerprinting the maternal metabolome using proton nuclear magnetic resonance spectroscopy. Endocrinology, Diabetes and Metabolism 4:00201. https://doi.org/10.1002/edm2.201
Shendure J, GJ Porreca, NB Reppas et al., 2005. Accurate multiplex polony sequencing of an evolved bacterial genome. Science 309:1728-32. https://doi.org/10.1126/science.1117389
Soni GV & A Meller, 2007. Progress toward ultrafast DNA sequencing using solid-state nanopores. Clinical Chemistry 53:1996-2001. https://doi.org/10.1373/clinchem.2007.091231
Sulmon C, J Van Baaren, F Cabello-Hurtado et al., 2015. Abiotic stressors and stress responses: What commonalities appear between species across biological organization levels? Environmental Pollution 202:66-77. https://doi.org/10.1016/j.envpol.2015.03.013
Taya N, N Katakami, K Omori et al., 2021. Evaluation of change in metabolome caused by comprehensive diabetes treatment: A prospective observational study of diabetes inpatients with gas chromatography/mass spectrometry-based non-target metabolomic analysis. Journal of Diabetes Investigation 12:2232-41. https://doi.org/10.1111/jdi.13600
Thomas R, JU Pontius, LB Borst et al., 2020. Development of a genome-wide oligonucleotide microarray platform for detection of DNA copy number aberrations in feline cancers. Veterinary Sciences 7:88. https://doi.org/10.3390/vetsci7030088
Thomford NE, DA Senthebane, A Rowe et al., 2018. Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. International journal of molecular sciences 19:1578. https://doi.org/10.3390/ijms19061578
Valouev A, J Ichikawa, T Tonthat et al., 2008. A high-resolution, nucleosome position map of *C. elegans* reveals a lack of universal sequence-dictated positioning. Genome Research 18:1051-63. https://doi.org/10.1101/gr.076463.108
Van der Oost R, J Beyer & NP Vermeulen, 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environmental Toxicology and Pharmacology 13:57-149. https://doi.org/10.1016/S1382-6689(02)00126-6
Verga JU, M Huff, D Owens et al., 2022. Integrated genomic and bioinformatics approaches to identify molecular links between endocrine disruptors and adverse outcomes. International Journal of Environmental Research and Public Health 19:574. https://doi.org/10.3390/ijerph19010574
Vucic EA, R Chari, KL Thu et al., 2014. DNA methylation is globally disrupted and associated with expression changes in chronic obstructive pulmonary disease small airways. American journal of Respiratory Cell and Molecular Biology 50:912-22. https://doi.org/10.1165/rcmb.2013-0304OC
Wang KC & HY Chang, 2018. Epigenomics: Technologies and applications. Circulation Research 122:1191-9. https://doi.org/10.1161/CIRCRESAHA.118.310998
Weber WA, HJ Wester, AL Grosu et al., 2000. O-(2-[18 F] fluoroethyl)-L-tyrosine and L-[methyl-11 C] methionine uptake in brain tumours: Initial results of a comparative study. European Journal of Nuclear Medicine 27:542-9. https://doi.org/10.1007/s002590050541
Wen PY, DR Macdonald, DA Reardon et al., 2010. Updated response assessment criteria for high-grade gliomas: Response assessment in neuro-oncology working group. Journal of Clinical Oncology 28:1963-72. https://doi.org/10.1200/JCO.2009.26.3541
Wester HJ, M Herz, W Weber et al., 1999. Synthesis and radiopharmacology of O-(2-[18F] fluoroethyl)-L-tyrosine for tumor imaging. Journal of Nuclear Medicine 40:205-12.
Wishart DS, T Jewison, AC Guo et al., 2012. HMDB 3.0-the human metabolome database in 2013. Nucleic Acids Research 41:801-7. https://doi.org/10.1093/nar/gks1065
Xu Y, J Pei & L Lai, 2017. Deep learning-based regression and multiclass models for acute oral toxicity prediction with automatic chemical feature

extraction. Journal of Chemical Information and Modeling 57:2672-85. https://doi.org/10.1021/acs.jcim.7b00244

Zhang B, B Li, XH Men et al., 2020. Proteome sequencing and analysis of *Ophiocordyceps sinensis* at different culture periods. BMC Genomics 21:1. https://doi.org/10.1186/s12864-020-07298-z

Zhang LW & NA Monteiro-Riviere, 2013. Use of confocal microscopy for nanoparticle drug delivery through skin. Journal of Biomedical Optics 18:061214. https://doi.org/10.1117/1.JBO.18.6.061214

Zhao Y, C Mir, Y Garcia-Mayea et al., 2022. RNA-binding proteins: Underestimated contributors in tumorigenesis. Seminars in Cancer Biology 86:431-44. https://doi.org/10.1016/j.semcancer.2022.01.010

Zhou ZH, 2022. Open-environment machine learning. National Science Review 9:123. https://doi.org/10.1093/nsr/nwac123

Zhu J, M Adli, JY Zou et al., 2013. Genome-wide chromatin state transitions associated with developmental and environmental cues. Cell 152:642-54. https://doi.org/10.1016/j.cell.2012.12.033

Ziegler A, C Faber, S Mueller et al., 2008. Systematic comparison and reconstruction of sea urchin (Echinoidea) internal anatomy: a novel approach using magnetic resonance imaging. BMC biology, 6:1-15. https://doi.org/10.1186/1741-7007-6-33

Ziegler A, S Mueller & T Bartolomaeus, 2010. Sea urchin (Echinoidea) anatomy revealed by magnetic resonance imaging and 3D visualization. In Proceedings of the 12th International Echinoderm Conference. https://doi.org/10.1201/9780203869543-c50