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Systems biology approaches to interpreting genomic data

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Abstract

Technological developments in genome-wide analysis have accelerated the generation of large, complex data sets characterizing human biology at the molecular level. Integration of data from different molecular levels holds great promise for gaining understanding of complex biological systems. Toxicogenomics aims to obtain a comprehensive mechanistic map of cellular processes that drive adverse outcomes. Such an integrated approach relies on combining various genome-wide profiles (DNA, RNA, protein, and metabolite) and linking these to functional endpoints to allow the identification of relevant biological pathways. Here, current strategies for generating multiomic data within the domain of toxicogenomics are highlighted, and current strategies for multiomic data integration are discussed.

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Systems biology, Toxicogenomics, Multi-omic data, Data integration.

1. Introduction

Studying the interactions between all components of a biological system overtime is also known as systems biology. The underlying rational for this approach is that functioning of a complex system cannot be predicted by its individual parts, even when these are fully understood. Applying a holistic approach that is focused on integrating data from multiple sources can improve understanding of the underlying mechanisms that determine the biological system characteristics. Such an approach requires an interdisciplinary team of investigators that can address the biological, technological, and computational aspects of such a comprehensive analysis. In addition, systems biology relies on the generation of robust and high-quality data sets from which statistical associations can be derived that point toward biological relationships (Figure 1).

2. Omic analysis

Genome-wide omic technologies play an important role in systems biology. The advancement in omic technologies such as DNA and RNA sequencing, protein mass spectrometry, and quantitative metabolomics has revolutionized the way biological systems are explored [1–3]. In the next section, currently applied omic strategies within the domain of toxicogenomics are described that generate a wealth of information providing a solid base for systems biology approaches.

2.1. Epigenomics

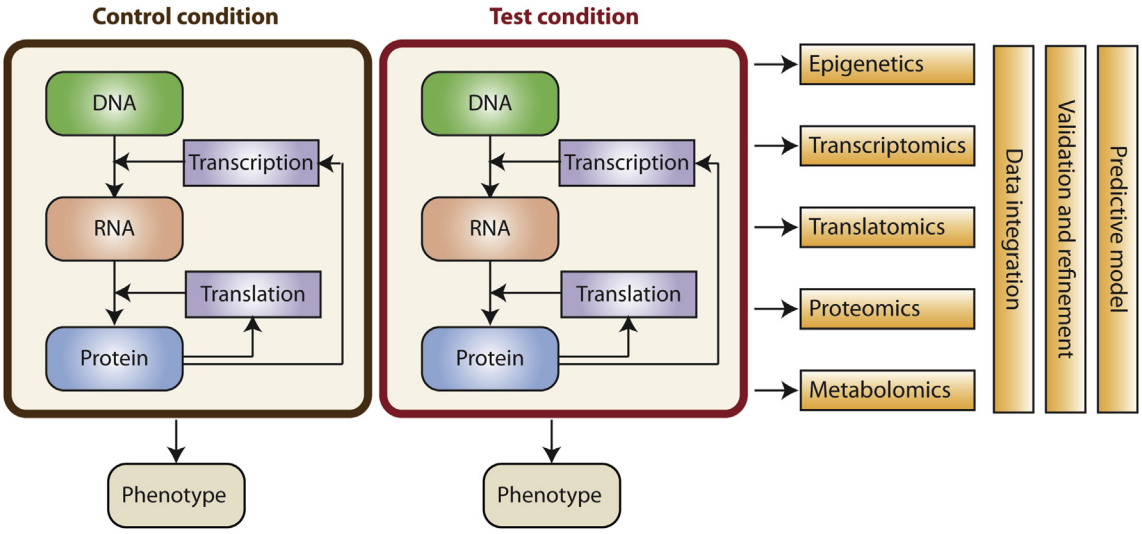
Analysis of the epigenome allows discovery of particular chromatin features at specific genomic loci. DNA methylation or post-translational modification of histone tails can control gene expression by altering chromatin compactness and DNA accessibility [4]. Over recent years, it has become evident that both histone modifications, as well as DNA methylation, are highly dynamic in supporting transcriptional responses [4,5]. Owing to their dynamic nature, studying these epigenetic marks is interesting for the identification of potential predictive biomarkers for toxicity and/or for gaining insights into the mechanisms underlying toxicity responses.

DNA methylation in the form of 5-methylcytosine (5-mC) is probably one of the best studied epigenetic marks in the domain of toxicogenomics. Several strategies have been developed over the years to map 5-mC patterns on a genome-wide scale [6]. Changes in 5-mC methylation have been associated with hepatotoxicity [7–9] and nephrotoxicity [10]. Those 5-mC DNA methylation patterns are reversible as they can be actively removed by oxidation to 5-hydroxymethylcytosine (5-hmC) through the ten-eleven translocation family of proteins and are thus of interest from a toxicological perspective [11]. Despite this, the number of studies investigating genome-wide alterations in 5-hmC patterns in response to xenobiotics remain limited [12–14].

2.2. Transcriptomics

Transcriptome analysis was traditionally performed by microarray analysis but is now almost completely replaced by next-generation RNA sequencing (RNAseq). Both

Figure 1



Systems biology for studying cellular function. Toxicogenomics is focused on identifying cellular mechanisms that are modulated in exposed cells as a function of time and dose. A versatile toolbox of 'omic' strategies enables the quantitative assessments of molecular events at multiple levels of biological organization. The big data sets that can be obtained from these analyses describe in high detail how substances affect cellular parameters. Integration of information across multiple biological layers of regulation is aimed at improving insights into the flow of information across these layers and aids in the generation of computer models that can be used to accurately predict an adverse outcome.

methods can be used to assess gene expression changes at the RNA level in response to substances with the intention to identify genes that are modulated by the exposure to these substances. A big advantage of transcriptomics lies in the highly reproducible gene expression profiles that can be generated in laboratories across the world [15]. RNAseq offers the advantage that it generates a richer data set compared with array technology including protein-encoding messenger RNA (mRNA), splice variants thereof, as well as, long noncoding RNA, circular RNA, and microRNA. These data sets can be used for the identification of new biomarkers for adverse outcomes or to gain insights into the molecular mechanism driving cellular responses upon drug exposure. Transcriptomics has been widely applied within the field of toxicogenomics. It can be used to identify gene signatures that distinguish two classes of substances [16–19]. Moreover, specific gene signatures can be used to predict the toxic features of new substances [20]. This is a powerful and potentially important application for hazard identification and risk assessment of the bulk of existing and new chemical entities. Several large databases, including DrugMatrix, diXa, and TG-GATES, exist that contain gene expression data from liver cells challenged with many different drugs at multiple doses and exposure times [21,22].

2.3. Translatomics

Functional interpretation of transcriptome data works under the assumption that gene-specific changes induced at the RNA level are propagated to the protein level. Despite notable success using this approach, it is more and more evident that changes in RNA expression are not always mirrored at the protein level or vice versa. Accumulating evidence suggests that mRNA-protein correlation on a genome-wide scale is low to absent [23–25]. This is not surprising, given the extensive post-transcriptional regulatory mechanisms mammalian cells possess [26]. To obtain a more direct proxy for protein abundance, RNAseq can be used to assess changes in mRNA translation. It is well known that, in response to stress, cells can rapidly alter the association between mRNA and ribosomes in a gene-specific manner and therefore their translation efficiency and protein synthesis. Several techniques, including polysome profiling and ribosome profiling, have been developed to assess global changes in mRNA translation [27,28]. Although this technology is widely used throughout the field of life sciences, its application in toxicogenomics is limited [29–31]. Yet, it might be essential for a more accurate interpretation of gene expression data. We have recently reported that primary mouse hepatocytes, exposed to the classical carcinogen benzo[a]pyrene, alter mRNA translation on a gene-specific basis [29]. BaP exposure affected the expression of a larger number of genes at the level of mRNA translation than at the level of mRNA abundance. This is relevant because cells that are partially unable to regulate mRNA translation in response to stress display reduced viability in response to BaP exposure [32].

More importantly, the translationally regulated genes belong to unique biological classes with distinct functions from transcriptionally responsive genes.

2.4. Proteomics

Proteins collectively determine cellular behavior or phenotype. Cells can adapt their phenotype by dynamically regulating protein abundance, modification, and localization in response to internal and external signals. It is not surprising that proteomics plays an important role in the field of toxicogenomics. Advances in mass spectrometry–based technologies have revolutionized proteomic analysis [2]. Thousands of proteins can be quantitated in a robust manner in biological samples [33]. Intracellular signaling relies heavily on post-translational modifications including phosphorylation, proteolysis, glycosylation, and ubiquitination. These modifications can be assessed using mass spectrometry (MS) by analyzing the difference in mass of a particular peptide that contains a known or suspected residue that may undergo post-translational modification [34–36]. Finally, proteomics can be used to study protein–protein interactions to get insights into protein complexes that are altered in response to a challenge, like for example, the recruitment of DNA repair machinery at the site of DNA damage [37] or the assembly of a transcription factor complex [38].

2.5. Metabolomics

Metabolomics can be applied to quantify multiple small molecules, for example, metabolites, such as amino acids, oligopeptides, sugars, fatty acids, or other products of cellular metabolic functions in cells or biological fluids [39]. Metabolite levels and their relative ratios are indicative of metabolic function. Dynamic changes in the production of metabolites upon a challenge reflect cellular dysfunction and are thus of interest from a toxicogenomics point of view [39,40]. Metabolomics can be used to identify hepatotoxic or kidney toxic substances [41–43]. Insights into the cellular mechanisms driving toxicity can be obtained from metabolomic studies [44,45]. Metabolomics was used to investigate variability in acetaminophen (APAP)-induced liver failure among five human donors. Variation in metabolite levels of hydroxyl/methoxy-APAP and C8H13O5N-APAP-glucuronide was associated with interdonor variation in an APAP-responsive gene signature. This work generates new clues to study interindividual susceptibility toward APAP [46].

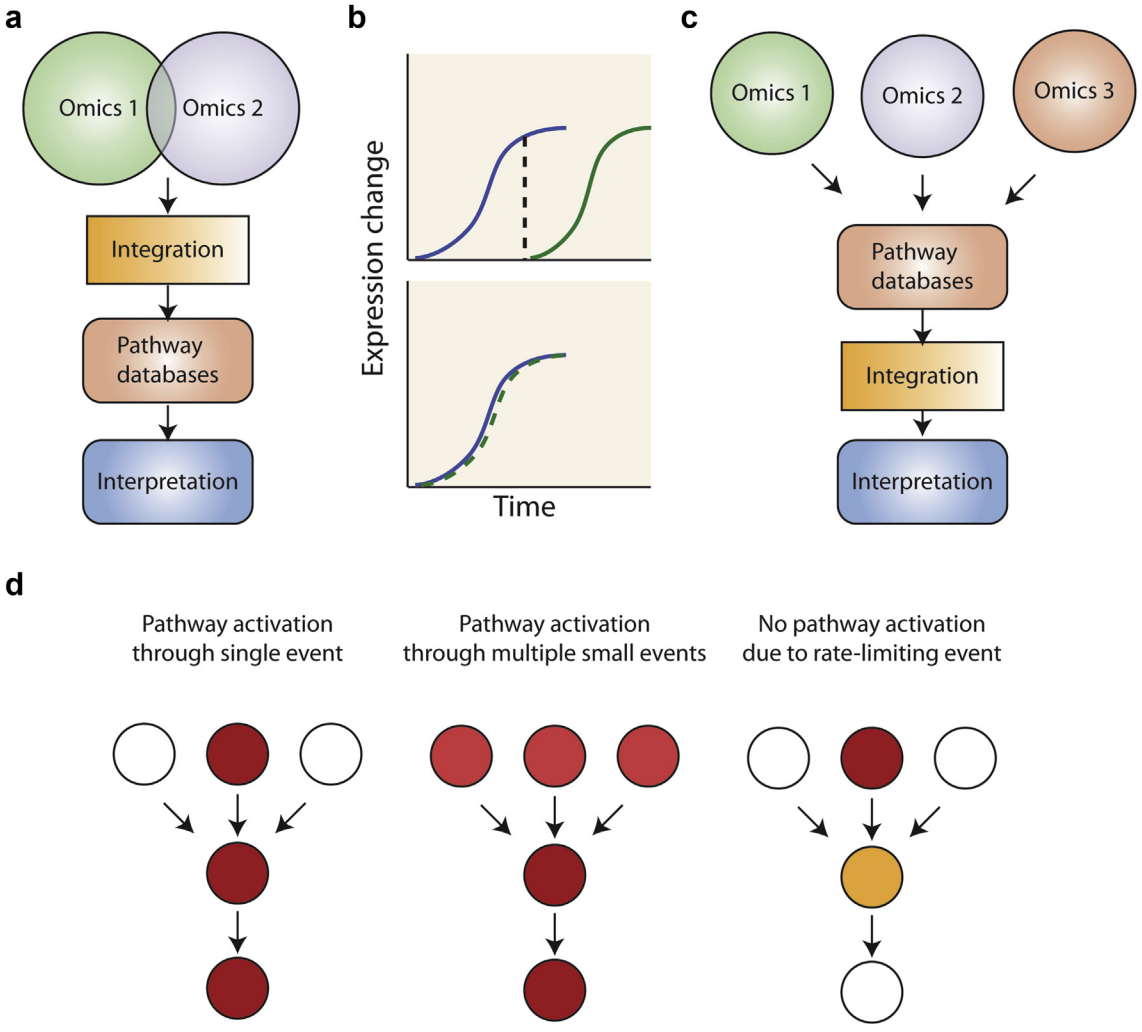
3. Multiomic data integration

3.1. Sequential integration

Integrative analysis of multiomic data sets is frequently performed in a sequential manner. In this fairly straightforward approach, findings from one omic data set are confirmed in a second or third omic data set representing a distinct biological layer (Figure 2a). This strategy allows biological validation and therefore increases the confidence level of the findings. Two or more types of data are required for this approach, for example, DNA methylation and gene expression data. Lists of differentially methylated regions are compared with lists of differentially expressed genes. This approach can identify genes that are coregulated at two or more biological layers. It can be used to discover genes that are transcriptionally activated upon exposure to a substance by promoter demethylation. Similarly, it can also be used to identify genes whose RNA expression is regulated via changes in 5-hmC decorations at promoter proximal regions [12]. This creates knowledge on how epigenetic changes are propagated to the mRNA expression level. A frequently applied strategy within the field of toxicogenomics is the combination of transcriptomics and metabolomics [47–49]. Integrating transcriptome and metabolome data is a powerful strategy in which phenotypic measurements in the metabolome can be linked to global changes in gene expression observed in the transcriptome. Yet it can be challenging because there is no direct connection between transcript and metabolite [47]. Using an integrative transcriptome and metabolome analysis of HepaRG cells exposed to polychlorinated biphenyl 126 (PCB126), Mesnage et al. [49] were able to identify novel biomarkers for toxicant-associated fatty liver disease. An integrative strategy can also be used to obtain a better impression of the metabolic state of cell models. Recently, Poupin et al. [50] generated transcriptomic and metabolomics data sets of HepaRG cells during differentiation, allowing a comprehensive assessment of the metabolic capacity of these human liver cells with minimum *a priori* knowledge.

A typical problem with this sequential approach is that the flow of information across several biological layers is dynamic. Therefore, changes that have occurred at a particular level may or may not have been propagated to the next biological layer. A certain offset in time is expected before changes from one biological layer are robustly detectable at a downstream layer. Therefore, a static, cross-sectional study of multiple omic layers could potentially fail to detect an event at a downstream biological layer, resulting in a false-negative conclusion (Figure 2b). Improved correlation between hierarchical layers could be improved by synchronizing time series obtained from distinct omic layers. This corrects time series that have similar characteristics but are locally out of phase. Cavill et al. [51] have previously used dynamic time warping (DTW) to improve integration of transcriptomic data with phenotypic data. This study investigated the effects of oxidative stress on gene expression over 9 time points using microarray data from human colon carcinoma cells exposed to H2O2 or menadione. On average, 85% of the genes identified by DTW were not found by standard correlation analysis. More importantly, combining DTW with overrepresentation analysis

Figure 2



Integration of 'Omics' data. **(a)** Integration and interpretation of multiomic data sets is frequently done using the intersection of 2 or more data sets as input data for comparison to general pathway databases. **(b)** Molecular events in different biological layers of regulation are often locally out of phase (changes in RNA expression are represented in blue and changes in protein in green). This can lead to a lack of correlation at a given time (stripped line). Correction of this phenomenon can increase the correlation between findings from one layer to another and improve the functional interpretation. **(c)** Integration of multiomic data sets at the pathway level is performed by identifying modulated pathways using each of the individual omic results. **(d)** Interpretation of omic data in the context of pathway activation is complicated. Induction of a single profound upstream event might trigger pathway activation (left panel). Similar pathway activation could be achieved through multiple smaller events (middle panel), whereas the presence of a rate-limiting event can block all upstream events and prevent pathway activation (right panel).

identified the 'oxidative stress' pathway, which correlates with the oxidative DNA damage (8-oxodG) present in the exposed cells. Standard correlation analysis failed to identify this pathway. This illustrates that important information can be lost during sequential data integration and that this can be prevented by using a suitable approach that considers time.

3.2. Integration at the pathway level

A second approach that is often used relies on integrating multiomic data sets at the pathway level (Figure 2c) [52]. Data integration at the pathway level offers the advantage that more biological regulation mechanisms are taken into account in contrast to strategies applying the intersection between data sets. A protein that is regulated in response to stress by altering protein stability (i.e. p53 in response to DNA damage) would be taken into account using this approach but would be disregarded in the sequential approach as it is not regulated at the level of RNA abundance. Therefore, this approach offers a richer, more comprehensive interpretation of the data sets but might also contain more noise. This approach takes advantage of existing knowledge databases that contain information of many

biological layers and interactions (epigenome, transcriptome, proteome, metabolome, and so on) [53]. This enables the possibility to perform some kind of integrative analysis even when only a single omic data set is available or on data sets generated by a sequential integration strategy. Pathway analysis has the ability to reduce large comprehensive data sets to smaller modules with biological meaning. However, interpretation of omic data in the context of pathway activation or deactivation remains challenging (Figure 2d). It is tempting to speculate that profound induction of an upstream event will result in pathway activation. On the other hand, subtle induction of several events in a pathway might have the same effect as a single large change. While a rate-limiting step in a pathway can lead to misinterpretation of upstream events. Pathway analysis is clearly a very powerful approach to generate new hypotheses based on omic data. Incomplete understanding of pathway schematics, including feedback and feedforward loops, rate-limiting events, interconnection between pathways, and the fact that pathway activation is highly dependent on cellular context makes it challenging to generate accurate predictions from omic data.

3.3. Data integration without prior knowledge

In contrast to the previously discussed approaches, several analysis strategies exist that do not rely on *a priori* knowledge. The big advantage of these types of strategies is that it potentially can discover new biology, whereas prior knowledge—based methods can confirm or refine existing understanding of biology at best. Within the field of toxicogenomics, these strategies have been mainly applied for classification of distinct chemical classes. In particular, transcriptome analysis is very successful for the detection of genotoxic carcinogens [16,17,54]. The study by Magkoufopoulou et al. illustrates that a transcriptomics-based *in vitro* assay can be of added value for predicting *in vivo* genotoxicity. Stratification of chemicals on Ames bacterial gene mutation assay prior to transcriptomic evaluation resulted in the best prediction. Improved accuracy and specificity and significant reductions in false-positive findings were observed compared with standard *in vitro* test battery, including the Ames assay. Another successful example is the transcriptomic biomarker TGx-DDI that can differentiate DNA damage-inducing substances from non-DNA damage-inducing substances [55]. This 64 gene signature can be used as a rapid and inexpensive method to evaluate genotoxic effects of test substances *in vitro* to predict *in vivo* genotoxicity.

These approaches have not been extensively applied to discover new biology underlying adverse outcomes mainly due to the fact that the number of data sets available for this type of analysis is limited. New and exciting opportunities for studying biological networks come from machine learning and deep learning approaches [56]. These analysis techniques are designed for building predictive models from multidimensional data sets. In machine learning, computers are programmed to learn patterns from large data sets using defined statistical and mathematical parameters. After the learning process is completed, the machine learning model is used to make predictions on new data. The accuracy of such a model is dependent on the size and quality of the training data set, as well as the complexity of the learning model. Overfitting (model is highly predictive on training data but not accurate on an independent data set) and underfitting (model poorly predicts training data) are frequently observed problems that can limit the predictiveness of a model generated by machine learning. Machine learning approaches have been successfully used in several domains of life sciences. Recently, the PsychENCODE consortium used integrative gene networks in combination with deep machine learning to predict psychiatric phenotypes from genotype and expression data from 1866 individuals [57]. Their deep learning module gave a ~6-fold improvement over traditional approaches illustrating the potential predictive power of deep learning approaches. Although there are still many challenges ahead before this technology can be routinely applied, machine learning holds great promise for generating predictive computational models and could play an important role in the context of toxicogenomics for predicting adverse outcomes.

4. Conclusion

It is clear that enhanced understanding of the dynamic flow of information across different biological layers of organization can effectively uncover biological pathways driving toxicity. Although multiomic data sets are being produced at a high pace in the field of toxicogenomics, data integration is not near its fullest potential. Most applied strategies require precise and detailed pathway descriptions including regulatory relationships and quantitative information. Currently, most pathways are annotated using data from various sources and cellular contexts and are therefore not accurate enough for many conditions. It is highly desirable to continue to expand knowledge databases with quantitative data and accurate reconstructions of pathways in a cell context—dependent manner. Continuous developments in generating standardized omic data sets and in computational approaches for integration these data sets will enable us to generate new testable hypotheses regarding the pathways underlying toxic responses. Ultimately, cycles of validation and refinement will lead to computational models that can predict adverse outcomes at an early stage.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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