

Epigenetic Mechanisms in Pharmacological Drug Discovery and Toxicity Studies to Predict the Pathogenesis of Human Diseases in the Era of Precision Medicine and of the Global Metabolic Disease Crisis

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Within global concerns of “metabolic diseases”, exposures to radiations, chemicals and microbial agents, the ineffective toxicological tests, the costly animal tests, governmental restrictions on animals for drug discovery and toxicity testing, new strategies are needed to reduce and treat these acute and chronic diseases. There is no universal acceptance of the mechanisms by which radiations, chemicals and microbial agents might contribute to the pathogenesis, prevention and treatment of human diseases.

Moreover, the emphasis on “Precision” or “Personalized” Medicine, together with the availability of sophisticated molecular technologies, is starting to generate tons of data, only to be analyzed by non-biologically-based algorithms. When humans are exposed to any pharmacological or toxic agent, there are only three mechanisms of responses: (a) mutagenesis of either “DNA repair errors” or “DNA replication errors;” (b) necrosis cytotoxicity, apoptosis, autophagy; and (c) epigenetic modification of gene expression at transcriptional, translational, or post-translation rates. Although mutagenesis can affect human health, only UV radiation is an effective point mutagen, while ionizing radiation is a powerful chromosomal mutagen, and viruses can be mutagens for insertion.

One needs to realize that there are three different cell types: stem cells, their progenitors and the terminally differentiated cells, each with different responses to these agents. While very controversial, it will be postulated that, even though many chemicals can induce oxidative stress, most natural and synthetic chemicals, that contribute to birth defects, cancer, cardiovascular immunological-reproductive or neurological diseases, act as epigenetic toxicants. Those drugs and chemo preventive agents seem to act epigenetically to prevent or treat various diseases.

The current use of human adult, organ specific stem cells, grown in 3-dimension, will be shown to discover new drugs and to test for toxicities, based on their upstream epigenetic effects on either secreted- or gap junctional -intercellular communication.

BACKGROUND:

Epigenetics is the study of DNA modifications which change the way genes express themselves. This fails to change an individual's underlying genetic code. When genes are switched on and off, epigenetic modifications effect.

DNA methyl transferases (DNMTs) add a methyl group (CH₃) to DNA, this addition acts as a ‘tag’ which can either activate the gene expression or (more commonly) repress it.

One of the well-characterized methylations of DNA is the addition of the methyl group at the 5-position cytosine nucleotide ring which results in 5-methyl-cytosine (5-mC) formation.

Epigenetic factors bind to ‘tails’ of the histone, which affects the degree to which DNA is wrapped around each histone. This affects the accessibility of genes, DNA that would normally be ‘hidden’ can become ‘visible’ and thus genes can be transcribed and expressed within that portion of DNA.

Genomic imprinting results in genes being expressed in a manner specific to parents of origin. Some genes are expressed from the maternally inherited allele, while others are expressed from the hereditary paternal allele.

Most human disorders present a loss or change in the identity of cells. It can be a transformation, degeneration or an immature state. Thus, drugs targeting the proteins involved in writing, reading, or erasing these epigenetic marks are attractive drug development targets. The DNA demethylating

agents and histone deacetylase inhibitors are approved for clinical use in leukemia and lymphoma subtypes in the field of oncology.

One major limitation is the biological space covered by specific-protein assays designed to evaluate a chemical's actions on specific enzymes or receptors. Acute toxicity could also involve more complicated or unspecific mechanisms. Toxic chemicals acting through non-specific mechanisms may not be identified in specific-protein assays or may be identified in multiple assays but with poor dose-response correlation, potentially confusing analyses. A final limitation is that certain specific-protein assays involve general processes that are common to several types of cells and thus may not themselves predict particular organ toxicity.

A main factor in all in vitro cell-based phenotypic assays is the choice of cell type. The general strategy is to seek to replicate different types of human cells; however, few data indicate, for example, that hepatocytes predict liver injury best, or that renal tubular cells diagnose renal injury best. Assays are performed with cells that are grown in a single layer, and these conditions ultimately provide only a poor approximation of in vivo tissue environments, cell types, and cell – cell interactions. In addition, for decades, immortalized and other cell lines have provided the cornerstone of cell-based assays, as they are convenient to obtain large numbers of cells in a standard state.

Measurement of assay results is another important technical consideration. Readings can be widely divided into those which well average the response of a number of cells in a tissue culture and those which evaluate individual cell behaviour, the latter sometimes referred to as high content assays. Whole-well readouts are most widely used and have the advantage of being fast and easy, and the generation of statistical metrics of assay performance and chemical activity is straightforward. High-content readouts have the advantage, in principle, of providing much more information.

Therefore, the assays typically combine measurements of acute cell lethality, cell proliferation, or cell metabolism and may represent several mechanisms simultaneously occurring. The longer an experiment is performed, the more it appears to affect cell proliferation effects unless it is performed on non-proliferating forms of cells. Short-term (1-hour) assays are also used but responsive cell injury tests need to be included. Some assays are based on ultraviolet (UV), since some chemicals may artificially interfere with assays based on UV or fluorescence. Luminescent readings have largely replaced UV and fluorescence-based assays in the pharmaceutical industry.

A variety of in vitro model systems were developed to study metabolism, including precision-cut tissue slices, subcellular fractions such as microsomal fraction, primary suspended cells, primary monolayers of cultured cells, continuous cell lines, immortalized primary cells, liver-derived cell lines that re-express biotransformation enzymes and genetically engineered cell lines. Recently, S9 encapsulation in hydrogel micro beads has been introduced into cytotoxicity assays as one way to reduce the leakage of potentially toxic microsomal lipid peroxides.

The greatest improvement required is the demonstration of a linkage of assay measurements with relevant toxicity mechanisms that quantitatively reflect a phenotype of in vivo toxicity in target cell types. This would also boost prediction of acute toxicity if the route of exposure is included in assay design. The committee believes that the specific routes of exposure are dermal and inhalation, and the majority of experiments are designed to model oral and intravenous exposures. The problems surrounding the route of exposure rise as assays become higher throughput and less metabolically competent.

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