Navigating the future of genotoxicity testing and the role of epigenetics

Due to the limitations of mutagenicity testing, a complete toxicity evaluation should include an evaluation of epigenotoxicity

- Gentoxicity is based on DNA structural changes, modifications, and mutations resulting from exposure to a compound, which can lead to mutagenic changes in DNA base pairs potentially leading to cancer.
- Epigenotoxic agents could cause significant modulation of the epigenome which mainly includes chromatin and DNA modifications resulting in altered gene expression.
- The development of epigenotoxicity studies would complement FDA required genotoxicity studies with a compound by evaluating changes in gene expression even when there are no DNA structural changes.

Genotoxicity and the Limitations of Mutagenicity Testing

Identifying agents that have long-term deleterious impact on health but exhibit no immediate toxicity is of prime importance in pharmaceutical development. It is well established that long-term toxicity of chemicals could be caused by their ability to generate changes in the DNA sequence through the process of mutagenesis. Several assays including the Ames test, and its different modifications, were developed to assess the mutagenic potential of chemicals (1-2). These tests have also been employed for assessing the carcinogenic potential of compounds. However, the DNA molecule contains within its chemical structure two layers of information: 1) the DNA sequence that bears the ancestral genetic information to produce RNA and protein molecules, and 2) the pattern of distribution of covalently bound methyl groups on cytosines in DNA which influence gene expression (3).

Genotoxicity and Epigenotoxicity

The epigenome is critical for long-term programming of all genome function and it serves as a mechanism for providing cellular identity during fetal development as well as post-fetal human development. In addition, the epigenome acts to adapt genome functions to environmental signal by reprogramming the genome to fit with anticipated environment. Stable disruption of epigenetic programs could lead to stable changes in phenotype particularly during gestation but also throughout life and is probably passed on to future generations similar to the consequences of genotoxic agents. Aberrations of epigenetic programs are involved in many human diseases including cancer, mental disorders, autoimmune disease, and diabetes. Remarkably, although there are acceptable tests to screen genotoxic agents, there are no regulatory requirements for screening for epigenotoxicity. Most compounds in use to date were never tested for epigenotoxic effects.

Epigenetics and DNA Methylation

DNA methylation patterns are generated by an innate program during gestation, but are attuned to the environment in utero and throughout life including physical and social exposures. DNA function and health could be stably altered by changing the state of DNA methylation on cytosines in genes by exposure to environmental agents without changing the sequence. The current FDA required genotoxicity and carcinogenicity screening tests (4-6) do not detect agents that have long-range impact on the phenotype (genetic expression) without altering the genotype (genetic sequence). The realization that long-range damage could be caused by pharmaceutical compounds without changing the DNA sequence has important implications on the way the safety of chemicals, drugs, and food are evaluated and broadens the scope of definition of toxic agents.

Impact of Epigenotoxic Agents on DNA and Chromatin Modifications

Epigenotoxic agents would cause significant modulation of the epigenome which mainly includes chromatin modifications and DNA modifications. There are several routes through which chemicals could alter epigenetic modifications. First, chemicals could either inhibit or possibly activate the enzymes that modify DNA or histones. Second, epigenetic modifications might be affected if chemicals inhibit the signaling pathways that regulate DNA methylation, for example during gestation or later in development after birth, by acting on microRNA or proteins that regulate histone and DNA modification enzymes. These agents could also influence pathways that regulate DNA and chromatin modification enzymes as well as proteins that translate epigenetic modification signals. Signaling pathways that regulate epigenetic processes are important in the brain and immune system as well as probably other tissues throughout life. As it is anticipated that epigenetic modifications might not immediately have phenotypic consequences or even simple functional readouts, the principal screen should be for agents that disrupt epigenetic programming. It is anticipated that agents that disrupt epigenetic modification significantly will potentially affect different functions of the genome at key points later in life and are therefore highly suspect. Additional prediction of potential damage could be achieved by analysis of the functional gene networks that are epigenetically altered by the agents. Another reasonable assumption is that although there are cell type and context specific idiosyncrasies of DNA and chromatin modification activities, the basic biochemical properties of the system are similar across tissues and across times and therefore agents that interfere with these fundamental biochemical properties will have phenotypic consequences downstream. Thus, developing screens that detect agents that interfere with fundamental biochemical properties of the epigenetic system should be considered as a first tier of protection from epigenotoxicity.

Epigenotoxicity and the Roles of the Methylome and Chromatin Modification

The molecular footprint of agents that affect epigenomic programming is the change in genome wide chromatin modification and DNA methylation. Therefore, assays that focus on alterations to the methylome and histone modification could reveal the extent of effect of the chemical on epigenetic programming and provide evidence

that the agent affects not only biochemical processes, but that it has functional consequences to the methylome. Analysis of the genome wide consequences of the treatment computationally would guide predictions on possible genome and physiological functions that might be affected. However, changes in the methylome will be impacted by cell type specificity and might restrict the effect of certain epigenetic agents to particular cell type or tissue class. Some important epigenotoxic agents might be missed by an assay based exclusively on examining DNA or chromatin modification that uses a restricted panel of cells that represent a restrictive set of time points in development. Using either in vivo models or a broad panel of cell types might escape this challenge. However, use of mouse or rat in vivo models might not reflect the particular repertoire of regulatory proteins and other regulatory molecules that exist in human cell types. Therefore, using only an in vivo model might possibly miss some potent epigenetic inhibitors or activators. Thus, the epigenotoxicity testing developed should consider evaluation in both in vitro human tissue cells and in vivo animal models to support use of the pharmaceutics in humans. Additional assays can be developed and validated for the detection of these types of genetic modifications in humans during clinical trials of investigational drugs so the public can be aware of these potential risks in commercially marketed products.

Epigenotoxicity – Differentiating Toxicity and Efficacy

To develop a battery of assays that will screen epigenotoxic reagents from early stages of nonclinical drug development to clinical trials in humans, it is critical to develop a sequence of assays that will follow the different stages of the drug development program. Comparable to genotoxicity screening, a sequence of in vitro assays, nonclinical animal studies, and clinical assays will be necessary to triage or at least limit the use of epigenotoxic drugs and agents at different stages of development. It will be important for FDA to establish an approved series of tests for regulators and the industry to implement to evaluate epigenotoxicity.

Some epigenetic agents could, however, have therapeutic benefits for certain disease conditions. Therefore, the methods developed should be used to distinguish agents with unknown epigenetic effects that could either result in safety concerns or possess therapeutic utility. Hence, while epigenetic drugs might be useful in certain diseases, the same agents might be toxic under normal circumstances, particularly during pregnancy. It is therefore critical to know whether a drug developed for non-epigenetic utilities has an epigenetic side effect which was not part of the intended mechanism of action. It should be noted that epigenetic processes act as a genomic long-term memory of transient exposures; therefore, transient exposure to a drug could result in long-term consequences. Thus, missing an unintended epigenetic effect could have long-term effects well beyond the time of treatment or exposure to the drug. This is one reason why epigenetic effects might be missed by most current in vitro toxicity screens as well as nonclinical animal and clinical human studies.

Another reason why epigenetic driven outcomes might go unnoticed is the fact that epigenetic effects reprogram genes, but since genes are expressed in different contexts of time, space, and external trigger; the long-term phenotypic impact might be lost if just immediate or short-term phenotypic consequences are examined. If the epigenetic consequences reprogrammed a response of a gene to trigger, this might be lost by study completion in the absence of triggers such as either stress or a bacterial or viral challenge. Two

epigenetically different animals might look the same under unchallenged conditions, but have dramatically different gene expression under challenge. In addition, epigenetic changes to the genome have a developmental context and seemingly innocuous exposures at specific times during development might have a phenotypic consequence later in life. It is therefore insufficient to measure standard “short-term” phenotypic consequences to rule out “long-term” and consequence-dependent epigenetic effects. This fact should also be considered when developing the overall approach for detection of epigenetic modifying agents. The focus on screening should be primarily on the ability of the agent to permanently alter epigenetic programs rather than an immediate genetic phenotype. A pipeline of epigenetic screens needs to be developed to shortlist epigenetic consequences that have significant potential phenotypic outcomes. Criteria for triaging agents at different points along the course of the pharmaceutical development pipeline should be established by FDA to ensure the safety of investigational and commercially available products used by humans.

Summary

Gentoxicity testing is based on DNA structural changes that lead to mutagenic changes in DNA base pairs which can alter gene expression and potentially lead to cancer. Epigenotoxicity complements genotoxicity, which also evaluates gene expression, yet it is based on methylation of DNA when there are no DNA structural changes. Epigenotoxic agents could cause significant modulation of the epigenome, which also includes chromatin modifications as well as DNA modifications. Based on the current understanding of epigenetics and limits of genotoxicity testing, it is incumbent on regulatory bodies to address the potential for epigenotoxicity testing throughout the stages of drug development.

References

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