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Progress in identifying epigenetic mechanisms of xenobiotic-induced non-genotoxic carcinogenesis

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Abstract

Determining the human relevance of structurally and functionally distinct non-genotoxic carcinogenic compounds that induce a diverse range of tissue-, gender-, strain- and species-specific tumours in animals remains a major challenge for toxicologists. Nevertheless, elucidating mechanisms of xenobiotic-induced tumours in animals can provide industry, environmental and regulatory scientists with valuable tools for cancer hazard identification and risk assessment. The discovery that aberrant epigenetic events frequently accompany genetic mutations in human cancers has stimulated efforts to deploy integrated epigenomic and transcriptomic profiling of xenobiotic-induced non-genotoxic carcinogenesis (NGC) in animal models, enabling enhanced mechanistic interpretation and novel early biomarker discovery. Recent advances in the mapping and functional characterization of mammalian tissue-specific epigenomes also provides new opportunities to characterize the cross-strain/-species chromatin architecture of non-genotoxic carcinogen effector genes and to predict their potential for modulation by xenobiotics in human tissue. Since xenobiotic-induced perturbations of gene regulation are intimately associated with the underlying DNA sequence, there is a need to integrate the impact of genotype on susceptibility to NGC. Furthermore, the potential association of xenobiotic target modulation with tumorigenic phenotypes can be assessed using genetic models and cancer genome resources. Finally, we discuss how epigenomic profiling may be used to critically assess the comparability and validity of cellular NGC models versus *in vivo*-derived tissue samples and some of key challenges associated with incorporating epigenetic mechanisms and biomarkers into cancer risk assessment.

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1. Introduction

Concerns regarding the appropriateness of extrapolating lifetime rodent carcinogenicity study findings to humans have been extensively reviewed [1–3]. If xenobiotic exposure in animals is found to be associated with either tumour induction or early indicators of neoplastic hazard, then a weight of evidence-based cancer risk assessment is generally recommended. A key contributing factor to the weight of evidence approach for xenobiotic-induced non-genotoxic carcinogenesis (NGC) is the determination of a mechanism or mode of action since this provides an entry point for subsequent assessments of potential human relevance [4–6]. A molecular basis for species-specific non-genotoxic carcinogenesis has been proposed for a number of compounds [4–10]. However, the diverse range of xenobiotic-induced tumour types that are typically observed in animal carcinogenicity studies, often exhibiting tissue-, gender-, strain- and/or species-specificities, make the determination of mechanism and assessment of potential relevance to humans very challenging. Furthermore, there is very little data on potential association of xenobiotic exposure with non-genotoxic carcinogenesis in humans due to the likely latency, very low incidence, and difficulty of deconvoluting environmental versus intrinsic factors for malignancy development. Some insight may be gained from somatic mutational signatures of human tumours that

are associated with known mutagenic exposures [11] but there will inevitably be overlap between intrinsic and extrinsic mechanisms [12]. Despite these challenges, there is a need for rigorous cancer risk assessment of xenobiotics to which humans are exposed including treatment with novel therapeutics [13] and occupational or environmental exposure to chemicals [3].

Characterizing the molecular mechanisms underlying xenobiotic-induced non-genotoxic carcinogenesis has great potential for providing industry, environmental and regulatory scientists with valuable tools for cancer hazard identification and risk assessment. This is exemplified by phenobarbital-induced hepatocarcinogenesis where Constitutive Androstane Receptor (CAR)-mediated stimulation of mouse hepatocyte proliferation represents a mode of action that has not been reproduced in human hepatocytes *in vitro* [14–16]. Since there is no clear evidence for phenobarbital-associated liver cancer risk in humans (based on epidemiological data from a large number of clinical studies including long-term therapeutic treatment of epileptics; [17]), CAR-mediated liver non-genotoxic carcinogenesis is not generally considered to be human-relevant [4,10]. Humanized rodent models in which mouse livers have been engineered to express human CAR supported proliferative responses and tumour promotion following exposure to PB [18–20]. In contrast, human hepatocytes did not support hyperplastic responses to the phenobarbital in chimeric mice with humanized liver [21]. It is noteworthy that the observed plasma phenobarbital exposures in these humanized models were comparable to those obtained in human subjects receiving therapeutic doses of this drug. Thus, understanding the opposing outcomes of these models will require further characterization of: i) quantitative exposure–response relationships; ii) the influence of human nuclear receptor-mouse gene regulatory protein interactions; iii) the influence of mouse host cellular environment on grafted human hepatocytes; and iv) comparability at the molecular, biochemical and cellular levels of engineered or grafted hepatocytes to human donor-derived liver tissue. Importantly, phenobarbital induces extensive changes in chromatin modification patterns across the regulatory regions of CAR target genes in mouse liver [22–24] and it is thus plausible that differences in the genetic and epigenetic architecture of phenobarbital effector genes play a role in determining species-specific susceptibility to CAR-mediated hepatocarcinogenesis.

Here we describe how recent advances in epigenetic regulation of the genome can be leveraged to provide new insights into molecular mechanisms of xenobiotic-induced non-genotoxic carcinogenesis, to identify early biomarkers and susceptibility factors, and to enable new approaches for assessing potential human relevance.

2. Leveraging recent advances in cancer epigenetics to enhance mechanistic interpretation and biomarkers of non-genotoxic carcinogenesis

Epigenetics describes mechanisms that operate in concert with the underlying DNA sequence to regulate gene expression and determine the overall phenotype of cell. Epigenetic marks include the methylation of DNA cytosine bases, post-translational modifications of histone proteins, nucleosome remodelling, and non-coding RNAs. These epigenetic marks are dynamically regulated by numerous enzymes and binding proteins that enable cells to read, write or erase chromatin modifications. Epigenetic formatting of the genome contributes to spatio-temporal patterns of gene expression and controls lineage choice, differentiation and cellular functions [25]. Epigenetic variation, together with genetic variation and meta-genomic variation, thus represents one of the key drivers of phenotypic variation in health and disease. The acquisition of cancer hallmarks such as sustained proliferative signalling and resistance to cell death [26] is facilitated by a combination of genome instability, mutation and epigenomic disruption [27]. Epigenetic perturbations associated with cancer aetiology and progression include widespread mutations in epigenetic regulatory proteins and aberrant expression of stem cell reprogramming genes [28]. Epigenetic mechanisms of carcinogenesis that are well characterized in humans include oestrogen exposure and breast cancer [29]. Importantly, from a toxicologic perspective, inflammatory responses to tissue injury and chronic exposure to environmental factors have been proposed as mechanisms for inducing cancer-predisposing epigenetic changes in vulnerable populations of somatic stem cells and progenitor compartments [30]. It is noteworthy that environmental influences on intermediary metabolism such as nutrient availability and utilisation can also result in tumour growth-promoting modifications of the epigenetic landscape by altering substrates and inhibitors of chromatin-modifying enzymes [30–34]. Epigenetic signatures of environmental exposure (e.g. to cigarette smoke) have already been identified in humans and are likely to exist for many other types of xenobiotic exposure in both humans and animals [35].

Together, these observations are stimulating efforts to investigate whether xenobiotic-induced perturbations of DNA methylation, chromatin modification, non-coding RNAs, and/or transcription factor accessibility contribute to non-genotoxic carcinogenesis [23,24,36–42].

3. Elucidating early epigenetic molecular indicators of non-genotoxic carcinogenesis

Integrated epigenomic and transcriptomic profiling of target tissues for xenobiotic-induced tumours represents a powerful approach for elucidating early