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The Future of Precision Medicine in Australia

Epigenetics

This input paper was prepared by Dr Tanya Medley and Professor Richard Saffery (Murdoch Children's Research Institute)

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1. Abstract

Epigenetics describes chemical modifications of DNA and its proteins that control gene expression, independent of the genetic code. Epigenetic processes that control gene expression are highly conserved through evolution but vulnerable to environmental factors. The epigenetic control of DNA is brutally altered in many diseases, including cancer. Analysis of these alterations helps elucidate the critical links between the environment and disease-causing changes in gene expression and can assist in the diagnosis of disease subtypes and the identification of effective treatments. To this end epigenetics has great potential to contribute to the advancement of clinical diagnostics and precision medicine.

Here we discuss the role epigenetics in human health and disease, its interaction with genetic and environmental influence, and potential for novel interventions in the clinic. Epigenetic discoveries are likely to contribute to improved strategies for diagnosis and treatments for a broad range of diseases. Unlike DNA mutations some epigenetic changes are reversible ultimately providing researchers with a platform to study how to slow or halt disease progression. Australia has been a key player in the field of epigenetics for decades but lags behind Europe and the United States in investing funding and the development of clear objectives for epigenetic therapeutics.

2. Introduction

Precision medicine. The National Institutes of Health (USA) define precision medicine as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person." In contrast NHMRC has defined personalised medicine as 'the capacity to predict disease development and influence decisions about lifestyle choices or to tailor medical practice to an individual. This includes targeted drugs and treatments based on a detailed understanding of the genetic basis of disease.' The common sentiment underlying of these definitions is the desire to specifically tailor interventions to an individual based on an understanding of the underlying pathogenic process associated with disease. In most cases such processes involve an interaction of genes and environment.

Epigenetics describes a suite of chemical modifications of DNA that regulate gene expression. While much research both here and overseas works to define the genetic architecture of disease, it is increasingly apparent that both genetic variations and environmental factors that interact with DNA to regulate gene activity (epigenetics) contribute to the risk and trajectory of human diseases.

To understand the relationship between the genetic code (DNA) and epigenetics one can image a book, where DNA represents all the letters and epigenetics makes the story readable by the introduction of

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spacing, punctuation and paragraphs. In our cells, if DNA is the blueprint of life, then epigenetics ensures that all our genes are turned on and off at the right time.

There are various terms used when discussing epigenetics. Epigenetics can be viewed as 'above DNA' and describes the factors that interact with DNA to regulate gene expression. Epigenetic mechanisms are a suite of distinct but related processes that occur to regulate the gene expression. An epigenetic variation refers to epigenetic changes at specific sites in the genome with the epigenome being sum of all epigenetic sites in the genome.

A large body of evidence has confirmed that epigenetic processes are sensitive to environmental influence. More recently, epigenetic profiles have been show to result from an interaction for both underlying genetic variation and environmental exposures. Thus, epigenetics is at the intersection of gene environment interactions underpinning a range of diseases.

2.1. Epigenetic mechanisms

All cells essentially contain the same DNA sequence and genes but not all genes are active at all times. Every different cell type (e.g. skin, liver, kidney and muscle) must express different genes at different times to ensure they form a specific cell type. There are many ways in which gene expression can be controlled with DNA methylation being one of the most studied mechanisms. DNA methylation is a mechanism used by cells use to lock genes in the 'off' position. DNA methylation is important not only during embryonic development and cell differentiation, but in genomic imprinting and chromosome stability. Given the vast processes in which methylation plays a role it's not surprising that errors in this mechanism are associated with a variety of devastating developmental conditions and human diseases.

2.2. Epigenetics in human disease

Typically genetic mutations and epigenetic errors both contribute to disease by disrupting the appropriate patterns of gene expression necessary for human health. Indeed the epigenome often shows considerable change with human diseases. This is particularly evident in human cancers of which all show gross changes in epigenetic profile relative to equivalent healthy tissue. There is also considerable interplay between different types of epigenetic mechanisms in cancer such that one (e.g. DNA methylation) can influence others to disrupt gene expression. While a control of gene expression is a complex process, in some instances harmful changes in gene expression can be restored to balance by reversing one type of epigenetic change.

Extensive research over many years has highlighted the 'layers' of epigenetic disruption present in human cancers. Where tested, many types of epigenetic disruptions are found to co-exist within human tumours, and appears to play a role in the transition to malignancy, tumour progression and metastases. A similar model is postulated to underlie many complex human diseases, though the level of genomic and epigenome variations are likely to be less pronounced.

Other classes of disease that have been linked to epigenetic variations include imprinting disorders such as Beckwith-Wiedemann, Silver Russell, Angelman and Prader-Willi syndromes and trinucleotide expansion diseases such as Fragile-X syndrome, Friedreich ataxia and Myotonic Dystrophy. More

recently, evidence is emerging (reviewed in (Michels et al. 2013)) to support an association between early life epigenetic variations and the onset of adult non-communicable diseases. In particular, epigenome wide variations have been associated with cardiovascular risk factors insulin and cholesterol levels (Hidalgo et al. 2014; Ma et al. 2015), type 2 diabetes (Dayeh et al. 2014; Toperoff et al. 2012), obesity (reviewed in (Van Dijk et al. 2015)) and elevated blood pressure (reviewed in (Udali et al. 2013)).

The emerging picture suggests that some epigenetic variants are predictors of disease thus offering the potential to identify those most at risk and whom will benefit from targeted intervention. The potential thus lies in the ability to translate this to enable identification of those at the highest risk of disease and to develop targeted personalised interventions.

3. Applications and opportunities

Analysis of epigenetic profiles contribute to understanding between environmental exposures, genetic variation and disease causing changes in gene expression. Ultimately scientists' and clinicians' vision is to translate this knowledge to aid diagnosis, predict onset of disease course and identify effective treatments in a personalised manor. To this end advancements in epigenetics may play an important role in advancing clinical diagnostics and treatments in precision medicine.

3.1. Epigenetics for improved diagnosis and risk stratification

Current technologies allow for accurate measurement of several epigenetic marks (places in the genome where epigenetic variations can occur) in human samples, particularly blood and saliva. Determination of epigenetic markers that reliably track disease stages will provide information on their disease progression and response to treatment on an individual patient basis. This has already been demonstrated in a range of adult cancers, where specific gene methylation signatures are used to inform treatment strategies, predictive long term outcome and monitor disease progression.

A comprehensive understanding of epigenetic markers may also provide an opportunity to improve risk prediction for other high burden non-communicable diseases as cardiovascular disease and diabetes. For example, childhood obesity measures can effectively predict onset of future cardiometabolic disease risk at the population level, but not at the individual level. Risk 'scores' based on genetics, clinical parameters, and epigenetic profile will be needed to accurately provide accurate individual risk evaluations.

3.2. Human therapies – dietary interventions

Perhaps the most widely studied area of environmental influence on epigenetics is the role of nutrition in establishing and maintaining a 'healthy' epigenome. Micronutrients such as folate and some vitamins (B_6 and B_{12}) play a key role in pathways that regulate epigenetic variations. Deficiencies have been linked to predisposition to several human diseases, while supplementation in animal models has capacity to reverse epigenetic variation associated with aberrant behaviour. At a broader level both under and over nutrition are linked to epigenetic disruption. Many foods contain chemicals with direct epigenetic modifying properties including chilli (curumin), carrots (retinoic acid), broccoli (sulforaphane), grapes (resveratrol) green tea (polyphenols) and soy (isoflavones), each of which inhibit or potentiate the

action of specific epigenetic processes. These compounds have either chemoprotective (cancer preventing) or cardioprotective properties, leading to speculation that a tailored 'epigenetic diet' could be appropriate for individuals identified at highest risk for such disorders.

3.3. Human therapies – pharmacologic interventions

One of the largest areas of clinical trials in the area of epigenetic therapies focuses on the inhibition of specific protein complexes responsible for aberrant epigenetic profiles in cancer (Dawson, Kouzarides & Huntly 2012). A second major class of epigenetic therapeutics specifically inhibits DNA methylation to reverse changes in gene expression associated with tumourogenesis.

Small molecule inhibitors are also showing promise outside the field of cancer. For example, a recent study of Prader Willi syndrome identified two small molecules that selectively inhibit one epigenetic mechanism that activated silenced genes associated with the disease (Kim et al. 2017). This is the first proof of principle that epigenetic-based therapies may play a role in treating this imprinting disorder.

3.4. Human therapies – epigenome engineering for targeted reversal of epigenetic errors

A potential use for recently emerged efficient gene editing technologies is to target specific epigenetic variations associated with disease. Such tools allow precise modification of epigenetic markers and will facilitate our understanding of their contribution to disease. The recent advent of gene editing approaches such as CRISPR–Cas9 (see section 5b, this chapter), offers potential beyond repairing damaging mutations in DNA. Gene editing technologies can be modified to allow for site-specific recruitment of epigenetic enzymes to add or remove specific epigenetic marks that control gene expression. This offers a novel approach to reverse gene silencing or activation associated with disease, independent of the DNA sequence. Several studies have now demonstrated the utility of these approaches in human cells, including silencing of specific genes in brain cells associated with pain stimulus, while preserving non-pathologic brain cell activity, in a model of chronic back pain (Stover et al. 2017).

In summary it is a long term goal to see individual epigenetic components, either alone or in combination, manipulated to restore a 'healthy epigenetic profile' leading to reactivation of critical genes and reversal of genome-wide epigenetic variations in disease. To realise this goal much investment is required to understand the underlying epigenetic disruption associated with individual diseases.

4. Gaps and the future

Australia has been a key player in epigenetics research for decades, having contributed several major discoveries in the field. Our skilled workforce, depth of clinical and scientific expertise, current focus on innovation (Innovation and Science Australia 2017) and rich patient and population-based cohorts collected over many years (many using Federal funding via NHMRC) are all in place to make important clinical contributions. However, resources are limited and objectives are not as strategically targeted as the United Stated and Europe. Epigenetics research is still carried out in largely disparate laboratories, several of who are actively competing against each other for resources and research outcomes. National

investment based on strategic priority areas identified through consultation and stakeholder agreement will be key to realising the potential for epigenetics in tailored/precision medicine in Australia.

5. Conclusion

The public health and economic burden of cancers and other non-communicable diseases continues to increase, despite successes of secondary and tertiary intervention in adulthood. Genetic risk plays a role, however many conditions are multigenic and unlikely to be amenable to only gene-editing approaches. In contrast, it is clear that specific epigenetic variants, potentially modifiable by dietary, pharmacologic or epigenome editing, represent a largely unexplored therapeutic avenue. The potential in cancers is increasingly clear, with many epigenetic drugs in various stages of clinical trials or in routine use. Only a small fraction of these are developed in Australia. The future for epigenetic monitoring and intervention to improve clinical care in non-cancer setting is largely underdeveloped, with no clear strategic priorities or research direction at a national level. What is clear is that Australia has the necessary expertise, infrastructure, patient and population cohorts necessary to fully explore this potential.

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