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EDITORIAL

Epigenetics and pharmacology

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Recent advances in the understanding of gene regulation have shown there to be much more regulation of the genome than first thought, through epigenetic mechanisms. These epigenetic mechanisms are systems that have evolved to either switch off gene activity altogether, or fine-tune any existing genetic activation. Such systems are present in all genes and include chromatin modifications and remodelling, DNA methylation (such as CpG island methylation rates) and histone covalent modifications (e.g. acetylation, methylation), RNA interference by short interfering RNAs (siRNAs) and long non-coding RNAs (ncRNAs). These systems regulate genomic activity 'beyond' simple transcriptional factor inducer or repressor function of genes to generate mRNA. Epigenetic regulation of gene activity has been shown to be important in maintaining normal phenotypic activity of cells, as well as having a role in development and diseases such as cancer and neurodegenerative disorders such as Alzheimer's. Newer classes of drugs regulate epigenetic mechanisms to counteract disease states in humans. The reports in this issue describe some advances in epigenetic understanding that relate to human disease, and our ability to control these mechanisms by pharmacological means. Increasingly the importance of epigenetics is being uncovered – it is pharmacology that will have to keep pace.

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Epigenetics

The term epigenetics describes genetic information that is 'beyond' or 'above' that information coded solely by our genetic code. Disease can be generated by a single base mutation [for example the deletion in cystic fibrosis patients of the phenylalanine (F) at position 508 of the cystic fibrosis transmembrane conductance regulator (CFTR)]. However, epigenetics may create disease in humans that have pristine un-mutated DNA. Exactly 38 years ago the first DNA sequence information was reported (Sanger *et al.*, 1977). Exactly 14 years ago, the human genome project reported the first complete sequence of the human genome (Lander *et al.*, 2001). Since then, scientists have struggled with the amount of DNA that was 'non-coding', initially referring to it as 'junk DNA'. Insults are usually given when someone is ignorant of that thing, and indeed this was true of the DNA that was not of immediately known function. This year sees the publication of parts of the epigenomic analyses to uncover epigenetic signatures that underlie developmental, healthy and diseased human physiological changes, which control the creation and maintenance of both healthy and diseased phenotypes of cells (Skipper *et al.*, 2015). Here we will consider the pharmacological manipulation of these processes and the potential such therapy may deliver.

Progress in pharmacology

Historically, there has always been pharmacology; however it wasn't always termed as such. From prehistoric use of plants as medicines, such as aspirin-like compounds from the bark of willow trees or other salicylate-rich plants in ancient Egyptian and Greek times, man has used drugs to control our physiology. Pain management is a good example of how Pharmacology has developed over time – from medicinal products with unknown ingredients that helped, through to chemically-synthesised purified compounds with known

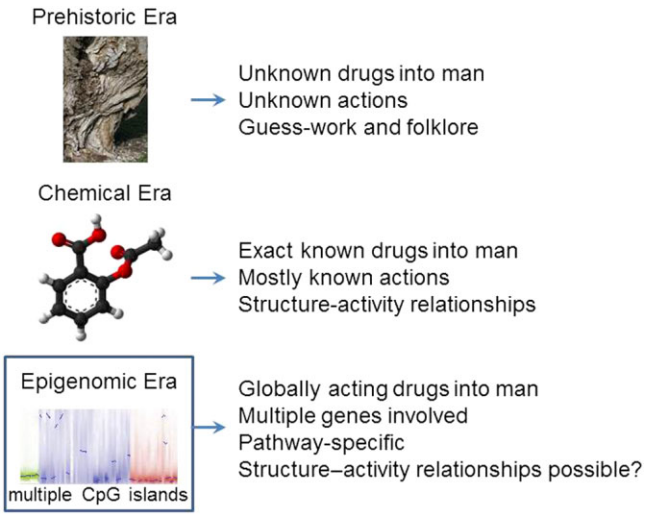

Figure 1

Diagram stressing the changes in our approach to Pharmacology and the advantages and disadvantages. Prehistoric era showing the bark of the willow tree. Chemical era showing the molecular structure of aspirin. The Epigenomic era demonstrates part of a gene's CpG analysis.

structure and activity – pharmacological science has developed our understanding and creation of therapeutically-useful compounds.

The epigenetic era

With recent improvements in understanding of the actual function of the entire genome, pharmacology has to modify itself further to think of tackling diseases not in the conventional 'drug-receptor' sense, but in a more 'global-response' sense. Drugs may not be designed to be as exact to a particular ligand or specific to a particular gene or protein subtype, they may indeed have to be able to be more broad-acting over a range of epigenetic large-scale events. These larger-scale epigenetic regulatory mechanisms may span more than one gene or family of proteins, they may in fact regulate large groups of genes (Figure 1). Importantly, the advantage to this approach is that often, the epigenetic variations can be the underlying cause of a particular disease, and simply targeting one protein of the multiple pathways involved may be futile. Diseases such as cancer often have many different mutation variations that are difficult to detect, predict and effectively treat, leading too often to relapse. Epigenetics of cancers may be the key to more effective treatments.

Cancer treatments are often at the forefront of drug design and development, as patients often have nothing to lose and may not have to deal with the long-term toxicological consequences of any new-to-the-market drug. Cancer itself is often treated with agents that have been around longer than we ourselves have. Recent advances in the development of cancer drugs have improved with more targeted agents (MacEwan, 2013). The cytidine analogue decitabine has long been used to treat myeloproliferative disorders and acute myeloid leukaemia. It is now known that the majority of decitabine's effects are at the epigenetic level rather than being incorporated into DNA to prevent cancerous cell growth (Rodríguez-Paredes, 2011). Decitabine, first synthesised in 1964 and under widespread use since the 1980s, is a DNA methyltransferase enzyme inhibitor and prevents DNA methylation, a type of epigenetic regulation of our genes. Furthermore, the agent vorinostat [also known as suberoyl anilide hydroxamic acid (SAHA)] is used in the treatment of T-cell lymphomas. Vorinostat and related compounds such as trichostatin A (TSA) are histone deacetylase (HDAC) inhibitors (HDACi) (Marks and Breslow, 2007). Similarly, valproic acid is another class of HDACi and as such is also an epigenetic drug by nature. Although first synthesised in 1882, valproic acid was known to inhibit induced convulsions in laboratory rats, and as such has been used for over a century to inhibit *petit mal* epileptic episodes. This common drug is an agent for regulating our epigenome. Similarly, many other common drugs may have significant effects to regulate the information contained in our epigenome, but as our knowledge of the epigenome is so recent and unknown, epigenetics of existing drugs may be far more critical than we presently realise.

RNAs small and large

The majority (approximately 60%) of the human genome is transcribed into RNA, however, only approximately 3% of that RNA gets coded into protein (i.e. via mRNA). Non-coding RNA (ncRNA) is RNA that is not translated into protein. Some ncRNAs, such as ribosomal RNA (rRNA) and transfer RNA (tRNA), are part of the protein synthesising machinery with well-defined roles. However, other ncRNAs have less well understood roles. MicroRNA (miRNA) is probably the best understood of the ncRNAs. It is known that miRNAs have an important role in regulating post-transcriptional mRNA function, to dampen down gene function. They are involved in a diverse range of cellular responses including development, homeostasis, metabolism, immunity, apoptosis and proliferation. It is thought that miRNAs have important regulatory roles in such cellular processes by 'fine-tuning' systems in healthy cells. They have been increasingly found to be important in pathological cellular conditions. As their target recognition is flexible, a single miRNA can affect a variety of genes to modulate entire pathways. Thus, miRNAs represent more 'global-regulators' that may be perfect in pharmacologically targeting certain human diseases and disorders. There are approaching 3000 different distinguishable species of miRNA and certain clinical trials are even using miRNA molecules themselves as pharmacological agents to treat a wide variety of conditions including primary liver cancer, glioblastoma and non-small cell lung cancer (NSCLC).

Less well understood are long ncRNAs which are over 200 bp long (as opposed to miRNAs which are processed to be around 22 bp long when mature). Long ncRNAs are also able to regulate gene transcription processes to suppress activity, but act in a manner more complex than miRNA action, by altering transcription factor function, RNA polymerase activity, DNA-RNA duplex formation and repression of the role of elongation factors. They are thought to have a role in ageing and chronic disease, including mis-processing of biochemical

such as that seen in Alzheimer's disease. Clearly, there is much more to non-coding 'junk' DNA than we imagined 14 years ago. The mechanisms, by which the epigenome functions, although complex, are also probably key to any successful pharmacological control that is needed for complex human diseases and disorders, which we also poorly understand pathologically.

Nutrition and the epigenome

There are several theories why a healthy balanced diet rich in fresh fruit and vegetables is good for your health. What may be undervalued as a mechanism by which our diet is able to stave off diseases of old age (cancer, cardiovascular diseases, dementia, etc.) is the role of epigenetics. Epigenetic modifications are heritable with simultaneous dynamics, plasticity and reversibility, and as such they are prone to changes upon external stimuli including diet. One of the strongest links between diet and the epigenome exists in one carbon metabolism where nutrients including methionine and folate are needed for synthesis of a methyl donor, S-adenosylmethionine (SAM) (Stefanska *et al.*, 2012). Any imbalances in the intake of these nutrients will impact the rate of methylation reactions in the human body and consequently DNA methylation patterns and histone methylation marks followed by alterations in chromatin and gene expression. Recent studies of the genome-wide DNA methylation profiles in different cancers reveal that hypermethylation and silencing of genes is only one side of the story. An almost equal number of gene promoters is activated following loss of DNA methylation marks (demethylation) (Stefanska *et al.*, 2011). These genes are putative drivers of carcinogenesis and metastasis. Using SAM as a tool to methylate and silence this group of genes appears to be a tailored way to impair metastasis (Shukeir *et al.*, 2015). The function of SAM in increasing DNA methylation has been also used in treatment of Alzheimer's disease with promising results in animal models (Scarpa *et al.*, 2006). Pharmacological intervention with SAM restores the epigenetic control of the β-secretase gene by hypermethylation and silencing followed by a decrease in formation of amyloid plaques.

The last decade of research has provided us with numerous pieces of evidence supporting the role of many other diverse groups of dietary compounds, particularly polyphenolics, in modifying the epigenetic marks. A few representatives of polyphenols, such as resveratrol, genistein, and curcumin, have been demonstrated to regulate gene expression through affecting several epigenetic components, from DNA methylation through histone modifications to microRNAs (Stefanska *et al.*, 2012). Apart from catechol compounds that reduce intracellular SAM pools available for DNA and histone methylation, polyphenols lack a direct link with the epigenetic machineries and the mechanisms underlying their epigenetic action are yet to be determined. The importance of epigenetic mechanisms in shifting the disease phenotype towards the healthy phenotype will be crucial to the adaptation of these compounds in pharmacological therapeutic approaches. With technological advances and next generation sequencing, the future will allow us to switch from studying candidate genes and single pathways to exploring a comprehensive genome-wide view of epigenetic effects of diets and isolated compounds and elaborating on underlying mechanisms of their epigenetic actions.

Challenges

As can be seen within some of the reviews in this issue, many challenges currently exist within the field of epigenetics. For example, invasive metastatic cancer may be more controllable by targeting DNA demethylation with synthetic (Cheishvili *et al.*, 2015) or naturally derived compounds (Shukeir *et al.*, 2015; Cheishvili *et al.*, 2015). Likewise, histone modification may be able to control cancerous phenotype (Simó-Riudalbas and Esteller, 2015). Interestingly, epigenetics may themselves modify how we respond to drugs altering our metabolic and pharmacokinetic handling of drugs themselves (He *et al.*, 2015). Thus miRNAs may be able to assist drug behaviour by altering its distribution of metabolism. Epigenetic drugs may assist not just in pharmacological therapy, but also in operations and pain management (Lirk *et al.*, 2015). Nutrition can help any pharmacological therapy (Remely *et al.*, 2015), indeed epigenetic mechanisms may assist in the mitochondrial adaptation necessary to counter type II diabetes (Henagan *et al.*, 2015).

As can be seen from this edition, there is still a lot of work that needs to be done to understand the epigenome workings, as well as scientists to use that epigenomic understanding to improve pharmacology and clinical therapy.

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