

## REVIEW

### Epigenetics in disease and cancer

Kong-Bung CHOO, *BSc(Hons), PhD*

*Faculty of Medicine and Health Sciences, University Tunku Abdul Rahman, Malaysia*

#### Abstract

Since the discovery of the double-helical structure of DNA, genetic regulation of gene expression has been well elucidated. More recently, another equally, if not more, important scheme of regulation of gene expression, called epigenetics, has emerged to explain the many biological observations that traditional genetic mechanisms have failed to decipher. Epigenetics is a discipline of study on the biological consequences of cellular alterations that do not involve nucleotide changes, as opposed to genetic mutations. Epigenetic changes are reversible and may lead to loss or gain of biological functions. The three most reported mechanisms of epigenetic regulation of gene expression involve changes in: (i) chromatin remodelling, (ii) DNA methylation and (iii) microRNA (miRNA). More importantly, many of the elucidated epigenetic changes are linked to the pathogenesis of human diseases and cancers. In this mini review, core concepts and basic experimental approaches in the study of epigenetic regulation of gene expression are briefly reviewed in relation to disease, with emphasis on cancer. This mini review also intends to highlight the fact that, besides genetics, epigenetics is now a discipline physicians and clinical research scientists can no longer ignore in their pursuit to understand disease and cancer and to develop new therapeutic strategies for treatment.

**Keywords:** Gene expression, epigenetics, microRNA, DNA methylation, chromatin modification, cancer, disease

#### INTRODUCTION

Life, if there is a bottom line, is about gene expression: when gene expression is relevant and appropriate, we live a healthy and normal life; when gene expression is in disarray and dysregulated, we get sick or develop cancers.

The classical genetic-molecular mode of regulation of gene expression is well established since the elucidation of the double-helical structure of DNA. There is then the Central Dogma in gene expression which states that: DNA is replicated using itself as a template, and the DNA template is used in transcription to generate messenger RNA (mRNA) which is subsequently translated into protein. Since then, another discipline in deciphering regulation of gene expression has emerged which is shown to play an equally, if not more, critical role in the control of the many biological processes. This discipline is collectively called epigenetics which studies the biological consequences of cellular alterations that do not involve nucleotide changes, as opposed to genetic mutations or single-

nucleotide polymorphism, SNP. As in genetic mutations, however, epigenetic changes also lead to loss or gain of biological functions. While genetic mutations are irreversible, epigenetic changes may be reversed. Presently, the three most well-defined areas of studies of epigenetic regulation of gene expression are (Figure 1A):

- (i) Chromatin remodelling
- (ii) DNA methylation (Figure 1B; see below for explanation)
- (iii) MicroRNA (miRNA) (Figure 1C; see below)

To expand the simplistic view of gene expression involving only transcription to translation first defined by the Central Dogma, epigenetics has immensely extended our repertoire of concepts in understanding gene expression to unprecedented levels. DNA methylation and chromatin remodelling add new dimension to gene regulation at the transcriptional level. At the post-transcriptional level, there is regulation by miRNA and, for this matter, an ever-expanding inventory of other

classes of noncoding RNAs (Figure 1A). From new understanding of epigenetic mechanisms come new insights on disease pathogenesis, and subsequently, prediction and treatment of diseases and cancers.

Owing to the vast amount of literature published on epigenetics, this review is only intended to briefly introduce to the readers core concepts of each of these topics with relevance to human diseases, particularly, to cancers. Chromatin remodelling will only be discussed briefly; more emphasis will be given to DNA methylation and miRNA involvement in human diseases and cancers. Reviews and literature cited here are meant for interested readers as the starting point for further reading on this important and ever evolving discipline.<sup>1</sup>

### 1. CHROMATIN REMODELLING IN HUMAN DISEASES

To rephrase a famous line in George Orwell's Animal Farm: All genes are created equal but some genes are more equal than others in expression. In the cell, there is an order of hierarchy but there is no democracy in gene expression.

The DNA that carries the entire set of genes in the human genome is wrapped around histones to form chromatin units which are the bricks of the superstructure called the chromosome. The intricately packaged DNA must be accessible to the molecular machineries of DNA replication and gene expression. Expression of a gene is, at the very initial stage, controlled by the accessibility of the promoter and regulatory sequences of

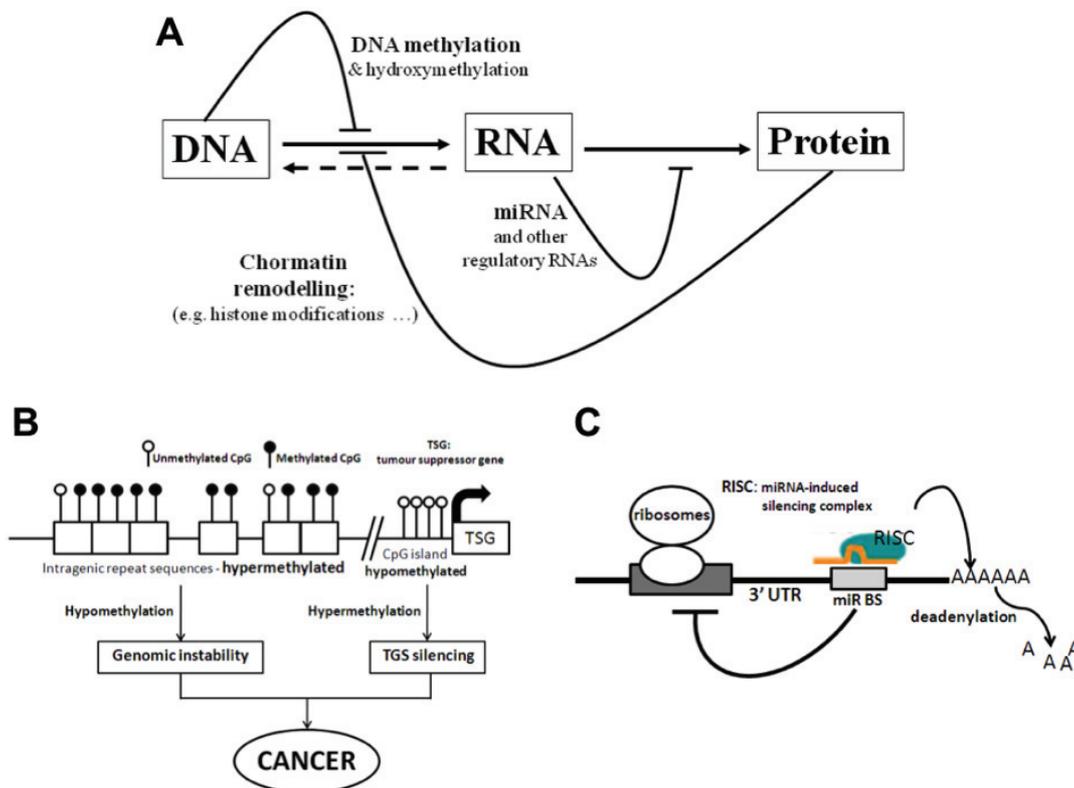


FIG. 1: Epigenetic regulation of gene expression. (A) Central Dogma revisited: Transcriptional and post-transcriptional epigenetic repression of gene expression by DNA methylation and hydroxymethylation, chromatin remodelling and miRNA. The classical genetic regulation pathway of transcription and translation is shown by solid arrows; the dashed arrow denotes reverse transcription. (B) A model of DNA methylation in tumorigenesis. Hypomethylation of the repeat-rich intragenic sequences and hypermethylation of tumour suppressor gene (TSG) are both associated with tumorigenesis. (C) miRNA regulation of a gene via targeting at miRNA binding site (miR BS) leading to deadenylation of mRNA and suppressed translation into protein.

the gene to the transcriptional machinery. And the accessibility is at the mercy of the local chromatin structure and configuration, the first key in controlling tissue- or development-specific expression patterns of many classes of genes. Histones and DNA-modifying enzymes are the basic components of the epigenetic machine that determine the permissiveness of the chromatin setting for gene expression. When the chromatin configuration is permissive, communication is established between the basic transcription machinery and the temporal- or spatial-specific transcription factors and co-regulators resulting in gene expression.

When under external physiological and biochemical assaults, histones may be chemically modified by processes including acetylation, methylation, phosphorylation and ubiquitination. When histones are modified, the chromatin is remodelled leading to changes in accessibility of gene(s) in the vicinity of the modified histones to transcription.<sup>2</sup> For examples, transcriptionally active regions may be abundant in histones that are modified in specific amino acids such as histidine (H) and lysine (K) residues in a H3K4me3 configuration; conversely, transcriptionally repressed regions are often defined by H3K27me3-modified histones.<sup>3</sup> Hence, for a gene, or a gene family, the expression status of which has been altered in a disease state, one may need to examine the first level of epigenetic regulation by examining histone modifications and altered chromatin structure.

## 2. DNA METHYLATION-REGULATED GENE EXPRESSION

In the mammalian genome, cytosine is a site for the addition of a simple methyl (CH<sub>3</sub>) group converting cytosine into 5'-methylcytosine, 5<sup>m</sup>C. And this event is DNA methylation. Since the number of 5<sup>m</sup>C sites in the human genome is not trivial, 5<sup>m</sup>C has been called the fifth base besides C, G, A and T.<sup>4</sup> To mention just in passing, a sixth base, 5-hydroxymethylcytosine, 5h<sup>m</sup>C, has also been discovered in 2009.<sup>5,6</sup> In the human genome, DNA methylation occurs almost exclusively on the cytosines of the CpG dinucleotides. Owing to uneven distribution of the (C+G) content in the human genome, pockets of CpG dinucleotides, called CpG islands (CGIs), are frequently found in the promoter and regulatory regions at the 5'-ends of many genes. It has been estimated that in the human genome, there are over 30,000 CGIs. Assuming the number of genes as ~20,000, each gene has a

share of 1.5 CGIs. That would mean that most, if not all, genes are regulated at one time or another, or in sickness or health, by some degree of DNA methylation. Why is DNA methylation important? This is because promoter methylation has direct consequences on gene expression.

### 2.1. Detection of 5mC in methylated DNA

DNA methylation may be detected at the single-gene or genomic levels. It suffices to point out here that genome-wide DNA methylation analysis in cancer and disease is commonly achieved by microarray chips supported by bioinformatics algorithms for meaningful analysis, and that genome-wide methylation analysis has wide implications in a more comprehensive understanding of the molecular mechanism of disease pathogenesis.<sup>7-9</sup> Here, only single-gene level detection of methylation changes is further discussed in some details.

Before CpG methylation analysis of a specific DNA fragment, most likely a promoter-regulatory sequence of a gene, it is first necessary to identify all possible CpG sites using one of the web-based algorithms available in the public domain. There are two most common approaches to perform DNA methylation analysis: Methylation-specific PCR (MSP) or bisulphite sequencing PCR (BSP). Both methods are based on a bisulphite reaction that converts unmethylated cytosines to "uracil" bases which, on polymerase chain reaction (PCR) amplification, are read as thymidines (T). On the other hand, the methylated cytosine in a 5<sup>m</sup>CpG dinucleotide is not modified by bisulphite treatment and remains as CpG. Hence, the hypothetical sequence 5'-AATC<sup>m</sup>CGTACT<sup>m</sup>CGCCTG-3', after bisulphite conversion, would be read as 5'-AATTCGTA<sup>m</sup>TCGTTG-3', where the *T*'s in italics are derived from the unmethylated C's whereas the methylated CpG remains CpG (underlined). The bisulphite-treated genomic DNA is then subjected to further analysis to identify the (un)methylated cytosines.

For MSP, two sets of PCR primers, both of which contain two or more CpG dinucleotides within the primer sequences, are designed: The U and M primer sets are for detection of unmethylated and methylated CpGs, respectively. If the promoter sequence under analysis is methylated, only the M primer set would generate a positive PCR band but not the U primers. Conversely, for unmethylated CpGs, only the U primer set would produce a positive PCR band but not the M primers. In the event that there

is a mixture of methylated and unmethylated genotypes, both the U and M primer sets would generate positive PCR bands. MSP is a convenient procedure applicable to simultaneous analysis of a large number of clinical samples, and has been widely used for elucidating the promoter methylation status of important genes in cancers.<sup>10</sup> To achieve clinical significance, paired cancer and adjacent non-cancerous biopsy samples are analysed to clearly determine how the methylation status of a gene promoter is related to up- or down-regulation of the gene in question in cancer cells.<sup>11</sup> In neurological diseases, in diabetes and metabolic syndromes and in many other human diseases, dysregulated expression of the disease-associated gene has also clearly been shown to be consequences of aberrant DNA hyper- or hypomethylation of the genes concerned.<sup>12-14</sup>

For accurate quantification of methylation levels, BSP is applied. BSP also involves bisulphite treatment of the genomic DNA, followed by PCR amplification as in MSP. In BSP, however, the PCR products are now cloned into an appropriate vector following which 5-10 independent clones are selected for DNA sequencing over the entire amplified sequence. In this way, all the CpG sites can be read for their methylation status. More importantly, each clone thus obtained represents a sequence from a chromosome and a cell; an analysis of 5-10 clones provides data on the methylation status from 5-10 different chromosomes and cells, and, hence, a better representation of the methylation status<sup>15, 16</sup>

## 2.2. Biological functions of DNA methylation

DNA methylation is intimately linked to gene reprogramming in embryonic development. On fertilisation in mammals, there is rapid demethylation of the genome in the preimplantation embryo up to the blastocyst stage in which the inner cell mass of a blastomere is now constituted of totipotent stem cells. On implantation, the embryonic genome undergoes methylation-driven reprogramming and differentiation. Appropriate and accurate methylation of the correct sets of genes and intragenic sequences is now essential for further development. Associated with embryonic-stage methylation is the phenotypic phenomenon called imprinting, which is defined as differences in gene behaviour between the paternal and maternal alleles. Some paternal genes are not expressed because of selective methylation of

the paternal allele inhibiting expression from this allele.

The most profound and well-characterised consequence of DNA methylation is its effects on gene expression. In general, DNA demethylation or hypomethylation is associated with increased, or up-regulated, gene expression, whereas DNA hypermethylation is linked to down regulation of genes. In other words, when a gene of interest is found to be down-regulated in, say, cancer cells, and no genetic mutations could be identified, one would then need to ask if the observed dysregulation is a consequence of hypermethylation of the said gene by performing MSP or BSP analysis.

What are the consequences of DNA hypermethylation? DNA hypermethylation promotes binding of proteins that induce histone modification and chromatin alterations affecting gene expression. On the other hand, transcription factors that regulate gene expression may be excluded from binding to methylated cis-acting elements altering the normal mode of gene expression. In cancer cells, DNA hypermethylation may lead to the repression of tumour suppressor genes (Figure 1B) whilst DNA hypomethylation is attributed to activation of an array of oncogenes.<sup>17</sup>

At the genomic level, however, a different scenario is found. The bulk of the human genome is known to be composed of repetitive sequences the biological significance of which is still being actively elucidated. In repeat-rich intragenic genomic sequences in normal cells, hypermethylation is the norm. If such sequences are hypomethylated in response to external physiological stresses, the chromosome becomes highly unstable with increased frequencies of mitotic recombination. In cancer cells, a combination of hypomethylation of the intragenic sequences and hypermethylation of tumour suppressor genes is commonly observed (Figure 1B).<sup>18</sup>

## 2.3. DNA methylation in cancer

The study of DNA methylation has led us to further understand the epigenetic contribution to the tumorigenesis process. It is now established that DNA methylation increases with aging and prolonged exposure to environmental carcinogens.<sup>19, 20</sup> Since methylation in different cells advances at different rates, different levels and patterns of DNA methylation start to develop in different cell populations on initiation to produce the inevitable heterogeneous cancer

cell population making treatment a lot more challenging.<sup>21</sup> Progressive accumulation and spread of DNA methylation in the promoters of tumour suppressor genes directly link DNA methylation with tumorigenesis. Genes that are hypermethylated in cancers have been abundantly reported.<sup>11,22</sup>

It has become clear that stem-cell like subpopulations exist in cancer tissues, and such cancer stem cells may yet be the major driving force in cancer pathogenesis.<sup>23, 24</sup> The one connection between stem and cancer cells is epigenetic aberrations: The innate epigenetic profile of normal embryonic and adult stem cells, or progenitor cells, may be the culprit in the evolution of subpopulations malignant cells when under physiological assaults.<sup>25</sup> Hence, epigenetic research in stem cells has direct relevance in understanding tumorigenesis.

Knowledge from our original understanding in embryonic reprogramming is currently being actively applied to stem cell research. For example, pluripotent stem cells, including induced pluripotent stem (iPS) cells, have now been shown to be more hypermethylated than their parental cells in general,<sup>26</sup> and methylation of pluripotency genes are crucial for proper cellular reprogramming.<sup>27</sup>

Recent researches have clearly established similarities in DNA methylation profiles in cancer and embryonic stem cells.<sup>28,29</sup> Methylation of promoters of germline genes occurs in the process of the development of embryonic stem cells to somatic stem cells; on differentiation from stem cells, there is further promoter methylation. Likewise in tumorigenesis, the genome of differentiated somatic cells undergoes hypomethylation to become precancerous cells which, when further inappropriate promoter methylation and accumulation of somatic mutations occur, are directed to become cancerous cells. In particular, the mesenchymal epithelial transition (EMT) process is common to both embryonic development and cancer invasion and metastasis.<sup>30</sup> Embryonic stem cells are deprogrammed (demethylated) cells that are undifferentiated and totipotent, and are potentially immortal and invasive through the EMT process as has been demonstrated in transplantation experiments. On the other hand, cancer cells are undifferentiated cells which are highly proliferative, immortal and are also invasive through EMT to become metastatic. Hence, the gene expression patterns of embryonic stem cells and cancer cells are highly similar.<sup>30,31</sup>

Besides cancer, abnormal DNA methylation profiles have also been linked to other diseases including diabetes and neurological diseases such as Alzheimer's disease just to name a couple of recent reports.<sup>32, 33</sup>

### 3. MICRORNA AND HUMAN DISEASES

The tale of microRNA (miRNA) should begin with an account on the RNA interference (RNAi) phenomenon. RNAi is a post-transcriptional process of gene silencing involving RNA. Two common mechanisms of RNAi have been well characterised:

- (i) Gene silencing by small interfering RNA (siRNA) which requires annealing of perfect complementary sequences between siRNA and the targeted mRNA. The consequence is mRNA degradation and shutdown of gene expression. siRNAs are easy to tailor-design in the laboratory and are widely used for studying the effects of silencing or knockdown expression of a gene in the laboratory.
- (ii) In miRNA-mediated gene knockdown or silencing, the miRNA sequence is only partial complementary with the target mRNA leading to deadenylation of the polyA tract of the mRNA and consequently translational repression.

Despite the historical event that miRNAs were first described in 1993 by Dr. Ambros's group,<sup>34</sup> and that the term RNA interference was only mentioned later in 1998,<sup>35</sup> the authors of the 1998 paper, Drs. Andrew Fire and Craig Mellow, were awarded the Nobel prize in Medicine and Physiology in 2006 for the discovery of RNAi and for proposing a molecular mechanism to explain gene silencing. However, Ambros's contribution was acknowledged in the acceptance speech of Drs. Fire and Mellow, linking up RNAi and miRNA.

#### 3.1. Basic features of microRNA

miRNAs are small (~18-25 nucleotides) endogenous single-stranded noncoding RNA species which are negative regulators in development and many physiological processes. As mentioned above, miRNA acts post-transcriptionally by targeting at one or more binding sites located, in most cases, in the 3'-untranslated region (3'-UTR) of its target mRNA (Figure 1C). A gene may be actively transcribed

into mRNA and yet gene expression is aborted due to little or no protein being produced as a consequence of miRNA repression. To date, over 866 miRNA genes have been identified in the human genome; since some miRNA genes may encode alternative mature miRNA species, over 1,100 mature mRNA species have been computed by the Sager miRNA database, v. 13.0. Approximately two-thirds of known genes are predicted to be regulated by one or more miRNA species; it would not be a surprise that in the years to come, most, if not all, genes are shown to be miRNA-regulated.

A miRNA gene is first transcribed into a precursor pre-miRNA which forms a hairpin structure with a double-stranded stem. In most cases, only the 5'-terminal leading strand becomes the mature miRNA whereas the opposite and complementary passenger strand is degraded. However, for some miRNA species, the passenger strand may also become the mature miRNA producing an alternative species. Owing to the large number of miRNAs that have been reported or predicted, and many more are to be anticipated, standardisation of miRNA nomenclature is essential (see the miRBase Registry at <http://microrna.sanger.ac.uk>). A few simple miRNA nomenclature rules are as follows: (i) miRNAs are assigned with letter prefixes to designate species, e.g. *hsa-miR-101* is for human (*homo sapiens*) and *mmu-miR-101* for mouse (*mus musculus*). (ii) miRNAs that are highly similar in sequences, i.e. differ by 1-2 nucleotides, are given lettered suffixes, e.g. *mmu-miR-10a* or *-10b*. (iii) Distinct miRNA genes that give rise to identical mature miRNAs are given numbered suffixes, e.g. *dme-mir-281-1* and *-281-2* genes both give rise to *dme-miR-281* in the fruit fly. miRNAs are often grouped into families of nearly identical isoforms, e.g. *hsa-miR-30* family contains more than 30 members including *-30a*, *-30b*, *30c*, *30d*, *30e* and others. The genes of some members of the same family may be physically clustered but other members may be located in different chromosomal sites. What defines miRNAs in the same family is that they share a highly similar seed or core sequence of 5-10 nucleotides.

### 3.2. Analysis of cellular levels of miRNA

To begin to study possible miRNA regulation of a gene, the mRNA sequence is first subjected to bioinformatics analysis to predict putative miRNA bind site(s). Prediction can now be

done using many free web-based algorithms, such as Targetscan, miRanda and miRNAmap. Bioinformatics prediction also includes the extent of conservation of the predicted miRNA binding sequence in other species, and the free energy for forming the miRNA-mRNA duplex. Once the binding site(s) for one or more miRNAs are predicted, experimental evidences for miRNA regulation is then sought by concurrent examination of the expression levels of the miRNA(s) and the mRNA and protein levels of the gene. miRNA regulation is supported by inverse relationship between the observed miRNA and protein levels, but not with the mRNA level of the gene. Subsequently, reporter assays are designed to analyse the effects of inclusion of the putative miRNA binding site and its mutagenesis in appropriate reporter vectors. Furthermore, one needs to assay and observe direct effects of the predicted miRNA on the biological functions of the target gene by ectopic over-expression of the miRNA, or repression of exogenous miRNA level by an miRNA inhibitor.<sup>36, 37</sup>

miRNA dysregulation has now been widely associated with diseases and cancers. One might wish to establish genome-wide miRNA expression profiles in normal and cancer cells to identify miRNAs that are differentially and aberrantly expressed in cancer cells. miRNA profiling may now be done by a number of microarray platforms using oligonucleotide chips.<sup>38-40</sup> miRNAs that have been identified in the high-throughput chips are then validated and further subject to bioinformatics-based analysis.

### 3.3. Biological functions of miRNA as key regulators

miRNAs are basically key negative regulators that are involved in almost all major physiological and biological processes, including early development, cellular proliferation and differentiation, cell cycle, apoptosis, signalling, metabolism, viral infection and tumorigenesis.<sup>41</sup> Haematopoiesis is also miRNA-regulated in normal blood cells; dysregulation of these key miRNAs may lead to haematopoietic cancers.<sup>42</sup> In many well-characterised signalling pathways, miRNAs are the central controllers. For example, *hsa-miR-145* is directly involved in the p53 signalling pathway by p53 activation and the consequential regulation a host of genes downstream of p53, including the oncogene *c-myc* and other cellular reprogramming factors.<sup>43</sup>

### 3.4. miRNA dysregulation in cancer

To understand contribution of miRNA to the tumorigenesis process, it is essential to identify dysregulated miRNA species in cancer by global miRNA expression profiling to identify miRNAs that are differentially expressed in cancer cells relative to the normal counterpart.<sup>44</sup> Based on such an approach, wide arrays of miRNAs have been mapped onto pathogenesis pathways common to many cancer types. Depending on whether a miRNA is up- or down-regulated in cancers, a cancer-associated miRNA is designated as oncogenic or tumour suppressive. Some miRNAs may seem to be associated with specific cancer types. Alternatively, the same miRNA or miRNA family may target different genes in different cancers. Conversely, a cancer-associated gene may be targeted by different miRNAs in different cancers.<sup>45-47</sup> In short, except for a small number of miRNAs that has repeatedly been shown to be dysregulated in different cancers, there is no clear set of rules to predict with confidence a set of miRNAs that may be involved in a particular cancer type.

At the chromosomal level, it has been clearly demonstrated that many onco-miRNAs are associated with chromosomal fragile sites.<sup>46,47</sup> When these chromosomal fragile sites are disrupted in the course of cancer initiation and progression, normal miRNAs functions are also disturbed, or physically lost in the event of chromosomal translocation or deletion, leading to profound effects to a wide spectrum of genes that are under the regulation of the perturbed or lost miRNA genes.

As has been briefly discussed above, studies in stem cell reprogramming have a direct bearing on understanding tumorigenesis. On introduction of a small number of specific pluripotency factors, somatic cells can be reprogrammed to become induced pluripotent stem cells, or iPS cells.<sup>48</sup> However, such reprogramming is only achieved with low efficiencies. More recently, it has been shown that a small set of miRNAs, when used in combination with the other previously established reprogramming factors, significantly increases the reprogramming efficiencies.<sup>43,49</sup> Subsequently, miRNAs have been identified that alone achieve reprogramming in the absence of the original pluripotency factors,<sup>50</sup> opening new opportunities for clinical applications by circumventing the need to use potentially oncogenic factors, e.g. c-myc, and also avoiding integration-induced genomic aberrations. The

finding of miRNA involvement in somatic cell reprogramming has also shed light on the course of tumorigenesis through shared pathways in the two processes. One such link is through the well-characterised tumour suppressor protein, p53.<sup>43</sup> It is now established that the p53 signalling pathway also plays a central role by activating *hsa-miR-145* that in turn suppresses expression of the reprogramming factors some major pluripotency factors.<sup>43</sup> Hence, down-regulating *hsa-miR-145*, and for this purpose, other p53-associated miRNAs, may relieve key pluripotency factors from *hsa-miR-145* suppression, accelerating reprogramming of somatic cells, or even cancer cells.

In developing miRNA-based cancer diagnostics, attempts are being made to establish unique miRNA signature profiles for each cancer type. Clinically more important is that miRNAs are detectable in body fluids, in particular in the blood, and body fluids are being tested in the attempt to develop diagnostic and prognostic markers for cancers.<sup>51</sup> Once a specific set of miRNAs and the associated targeted genes have been identified and validated for a particular cancer, therapeutic strategies based on miRNA-mediated correction of aberrant transcript levels of genes may then be developed.<sup>52</sup>

## 4. EPIGENETIC THERAPY OF DISEASE AND CANCER

Understanding the molecular mechanisms involved in the initiation and maintenance of epigenetic abnormalities in cancer has great potential for translational purposes in terms of epigenetic biomarker development and for pharmacological targeting aimed at reversing cancer-specific epigenetic alterations. Epigenetic drugs that can reverse DNA methylation and aberrant histone modifications in cancers are being reported with increasing frequencies. In general, epigenetic alterations in cancer cells can be reversed by inhibition of DNA methylation using inhibitors of DNA methyltransferases, the methylation enzyme that adds the methyl group to the cytosine, and also inhibitors of histone deacetylation to reverse the detrimental effects of chromatin remodelling.<sup>53</sup> As DNA methylation inhibitors, the FDA-approved 5-azacitidine and 5-aza-decitabine are being clinically tested for treatment of myelodysplastic syndromes and other hematological malignancies such as acute myeloid leukemia and chronic myeloid leukemia.<sup>54</sup> At the same time, small-molecule

non-nucleoside compounds are also being developed as alternative epigenetic drugs to reverse hypermethylation by blocking expression of the DNA methylation enzymes.<sup>55,56</sup>

On therapeutically reversing aberrant chromatin modifications, the histone modifier enzyme, histone deacetylase (HDAC), is being targeted. Histone deacetylases remove acetyl groups from the histones, and are agonists of histone acetyltransferases in chromatin remodeling relevant to gene expression. The role of histone deacetylases in cancer initiation and progression, and in other human diseases, has been well described.<sup>57</sup> In cancer cells, HDAC inhibitors are used to re-establish normal histone acetylation patterns through reactivation of silenced tumour suppressor genes to achieve antitumorigenic effects such as growth arrest, apoptosis and induction of differentiation.<sup>58</sup> As an example, the HDAC inhibitor, suberoylanilide hydroxamic acid, has now been clinically approved for treatment of T-cell cutaneous lymphoma.<sup>56</sup> For other human diseases, histone deacetylation has also been successfully targeted for fibrotic disorders.<sup>57</sup>

Interestingly, but not unanticipated, combined use of DNA methylation and HDAC inhibitors has been shown to be more effective than individual treatment. Furthermore, epigenetic drugs are also being tested in combination with chemo-, radio- or hormonal therapies to achieve optimal therapeutic effects on cancer.<sup>59,60</sup> Successes have also been reported in the concerted use of DNA methylation inhibitors and activation of miR-127 to down-regulate the BCL6 oncogene.<sup>61</sup> Chemically synthesised tumour suppressor miRNA mimics are able, in theory, to selectively repress oncogenes in tumours.<sup>62</sup> The bottleneck with miRNA therapy at present is the lack of an efficient miRNA delivery system to cancer cells.

## CONCLUDING REMARKS

Epigenetics has added a new dimension to the understanding and treatment of human diseases and cancers. Epigenetics is now a discipline physicians and clinical research scientists can no longer ignore, or be ignorant of. Fundamental and translational researches now need to work hand-in-hand to achieve deeper understanding of global epigenetic alternations associated with diseases before diagnostic and prognostic biomarkers and better therapeutic strategies may be developed. Appreciating the role of cancer stem cells, and the contributions of epigenetic

changes in these cells, may yet expand our field of vision in the development of effective epigenetic drugs aimed at healing the disarrayed epigenome in the cancer cell.

## REFERENCES

1. Caffarelli E, Filetici P. Epigenetic regulation in cancer development. *Front Biosci* 2011;17:2682-94
2. Chandrasekharan MB, Huang F, Sun ZW. Histone H2B ubiquitination and beyond: Regulation of nucleosome stability, chromatin dynamics and the trans-histone H3 methylation. *Epigenetics* 2010;5:460-8
3. Hargreaves DC, Crabtree GR. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res* 2011;21:396-420
4. Lister R, Ecker JR. Finding the fifth base: genome-wide sequencing of cytosine methylation. *Genome Res* 2009;19:959-66
5. Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxy-methylcytosine in mammalian DNA by MLL partner TET1. *Science* 2009;324:930-5
6. Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. *Science* 2009;324:929-30
7. Fernandez AF, Assenov Y, Martin-Subero JI, et al. A DNA methylation fingerprint of 1628 human samples. *Genome Res* 2011; Jul 12, 2011 [Epub ahead of print]
8. Hinoue T, Weisenberger DJ, Lange CP, et al. Genome-scale analysis of aberrant DNA methylation in colorectal cancer. *Genome Res* 2011, Jun 9, 2011 [Epub ahead of print]
9. Gupta R, Nagarajan A, Wajapeyee N. Advances in genome-wide DNA methylation analysis. *Biotechniques* 2010;49:iii-xi
10. Shames DS, Minna JD, Gazdar AF. Methods for detecting DNA methylation in tumors: from bench to bedside. *Cancer Lett* 2007;251:187-98
11. Chen ST, Choo KB, Hou MF, et al. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. *Carcinogenesis* 2005;26:1241-6
12. Villeneuve LM, Natarajan R. Epigenetic mechanisms. *Contrib Nephrol* 2011;170:57-65
13. Sugii S, Evans RM. Epigenetic codes of PPARgamma in metabolic disease. *FEBS Lett* 2011;585:2121-8
14. Ling C, Groop L. Epigenetics: a molecular link between environmental factors and type 2 diabetes. *Diabetes* 2009;58:2718-25
15. Hsu MC, Huang CC, Choo KB, Huang CJ. Uncoupling of promoter methylation and expression of Period1 in cervical cancer cells. *Biochem Biophys Res Commun* 2007;360:257-62
16. Tokita T, Maesawa C, Kimura T, et al. Methylation status of the SOCS3 gene in human malignant melanomas. *Int J Oncol* 2007;30:689-94
17. De Smet C, Lorient A. DNA hypomethylation in cancer: Epigenetic scars of a neoplastic journey. *Epigenetics* 2010;5: doi:11447[pil]
18. Robertson KD. DNA methylation and human disease. *Nat Rev Genet* 2005;6:597-610

19. Aguilera O, Fernandez AF, Munoz A, Fraga MF. Epigenetics and environment: a complex relationship. *J Appl Physiol* 2010;109:243-51
20. Christensen BC, Houseman EA, Marsit CJ, et al. Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. *PLoS Genet* 2009;5:e1000602
21. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002;3:415-28
22. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? *Nat Rev Cancer* 2006;6:107-16
23. Soltanian S, Matin MM. Cancer stem cells and cancer therapy. *Tumour Biol* 2011;32:425-40
24. Alison MR, Islam S, Wright NA. Stem cells in cancer: instigators and propagators? *J Cell Sci* 2010;123:2357-68
25. Tsai HC, Baylin SB. Cancer epigenetics: linking basic biology to clinical medicine. *Cell Res* 2011;21:502-17
26. Nishino K, Toyoda M, Yamazaki-Inoue M, et al. DNA methylation dynamics in human induced pluripotent stem cells over time. *PLoS Genet* 2011;7:e1002085
27. Ohi Y, Qin H, Hong C, et al. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat Cell Biol* 2011;13:541-9
28. Topczewska JM, Postovit LM, Margaryan NV, et al. Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. *Nat Med* 2006;12:925-32
29. Zilberman D. The human promoter methylome. *Nat Genet* 2007;39:442-3
30. Kang Y, Massague J. Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* 2004;118:277-9
31. Hendrix MJ, Seftor EA, Seftor RE, et al. Reprogramming metastatic tumour cells with embryonic microenvironments. *Nat Rev Cancer* 2007;7:246-55
32. Coppieters N, Dragunow M. Epigenetics in Alzheimer's Disease: A Focus on DNA Modifications. *Curr Pharm Des* 2011;doi:BSP/CPD/E-Pub/000707[pii]
33. Bell CG, Finer S, Lindgren CM, et al. Integrated genetic and epigenetic analysis identifies haplotype-specific methylation in the FTO type 2 diabetes and obesity susceptibility locus. *PLoS One* 2010;5:e14040
34. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* 1993;75:843-54
35. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 1998;391:806-11
36. Webster RJ, Giles KM, Price KJ, et al. Regulation of epidermal growth factor receptor signaling in human cancer cells by microRNA-7. *J Biol Chem* 2009;284:5731-41
37. Mohri T, Nakajima M, Takagi S, Komagata S, Yokoi T. MicroRNA regulates human vitamin D receptor. *Int J Cancer* 2009;125:1328-33
38. Oberg AL, French AJ, Sarver AL, et al. miRNA expression in colon polyps provides evidence for a multihit model of colon cancer. *PLoS One* 2011;6:e20465
39. Git A, Dvinge H, Salmon-Divon M, et al. Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. *RNA* 2010;16:991-1006
40. Schetter AJ, Leung SY, Sohn JJ, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA* 2008;299:425-36
41. Bueno MJ, Malumbres M. MicroRNAs and the cell cycle. *Biochim Biophys Acta* 2011;1812:592-601
42. Zimmerman AL, Wu S. MicroRNAs, cancer and cancer stem cells. *Cancer Lett* 2011;300:10-9
43. Sun X, Fu X, Han W, Zhao Y, Liu H. Can controlled cellular reprogramming be achieved using microRNAs? *Ageing Res Rev* 2010;9:475-83
44. Jiang J, Lee EJ, Gusev Y, Schmittgen TD. Real-time expression profiling of microRNA precursors in human cancer cell lines. *Nucleic Acids Res* 2005;33:5394-403
45. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. *Dev Biol* 2007;302:1-12
46. Durkin SG, Glover TW. Chromosome fragile sites. *Annu Rev Genet* 2007;41:169-92
47. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc Natl Acad Sci USA* 2004;101:2999-3004
48. Yamanaka S. A fresh look at iPS cells. *Cell* 2009;137:13-7
49. Li Z, Yang CS, Nakashima K, Rana TM. Small RNA-mediated regulation of iPS cell generation. *EMBO J* 2011;30:823-34
50. Subramanyam D, Lamouille S, Judson RL, et al. Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. *Nat Biotechnol* 2011;29:443-8
51. Mitchell PS, Parkin RK, Kroh EM, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA* 2008;105:10513-8
52. Slaby O, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. *Mol Cancer* 2009;8:102
53. Sharma S, Kelly TK, Jones PA. Epigenetics in cancer. *Carcinogenesis* 2010;31:27-36
54. Plimack ER, Kantarjian HM, Issa JP. Decitabine and its role in the treatment of hematopoietic malignancies. *Leuk Lymphoma* 2007;48:1472-81
55. Datta J, Ghoshal K, Denny WA, et al. A new class of quinoline-based DNA hypomethylating agents reactivates tumor suppressor genes by blocking DNA methyltransferase 1 activity and inducing its degradation. *Cancer Res* 2009;69:4277-85
56. Cortez CC, Jones PA. Chromatin, cancer and drug therapies. *Mutat Res* 2008;647:44-51

57. Pang M, Zhuang S. Histone deacetylase: a potential therapeutic target for fibrotic disorders. *J Pharmacol Exp Ther* 2010;335:266-72
58. Carew JS, Giles FJ, Nawrocki ST. Histone deacetylase inhibitors: mechanisms of cell death and promise in combination cancer therapy. *Cancer Lett* 2008;269:7-17
59. Sebova K, Fridrichova I. Epigenetic tools in potential anticancer therapy. *Anticancer Drugs* 2010;21:565-77
60. Kristensen LS, Nielsen HM, Hansen LL. Epigenetics and cancer treatment. *Eur J Pharmacol* 2009;625:131-42
61. Saito Y, Liang G, Egger G, et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell* 2006;9:435-43
62. Friedman JM, Liang G, Liu CC, et al. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. *Cancer Res* 2009;69:2623-9